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CDP-Choline: Pharmacological and Clinical Review

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SUMMARY

Cytidine 5´-diphosphocholine, CDP-choline or citicoline, is an essential intermediate in the biosynthetic pathway of the structural phospholipids of cell membranes, especially in that of phosphatidylcholine. Upon oral or parenteral administration, CDP-choline releases its two principle components, cytidine and choline. When administered orally, it is absorbed almost completely, and its bioavailability is approximately the same as when administered intravenously. Once absorbed, the cytidine and choline disperse widely throughout the organism, cross the blood-brain barrier and reach the central nervous system (CNS), where they are incorporated into the phospholipid fraction of the membrane and microsomes. CDP-choline activates the biosynthesis of structural phospholipids in the neuronal membranes, increases cerebral metabolism and acts on the levels of various neurotransmitters. Thus, it has been experimentally proven that CDP-choline increases noradrenaline and dopamine levels in the CNS. Due to these pharmacological activities, CDP-choline has a neuroprotective effect in situations of hypoxia and ischemia, as well as improved learning and memory performance in animal models of brain aging. Furthermore, it has been demonstrated that CDP-choline restores the activity of mitochondrial ATPase and of membranal Na^+/K^+ ATPase, inhibits the activation of phospholipase A_2 and accelerates the reabsorption of cerebral edema in various experimental models. CDP-choline is a safe drug, as toxicological tests have shown; it has no serious effects on the cholinergic system and it is perfectly tolerated. These pharmacological characteristics, combined with CDP-choline's mechanisms of action, suggest that this drug may be suitable for the treatment of cerebral vascular disease, head trauma of varying severity and cognitive disorders of diverse etiology. In studies carried out on the treatment of patients with head trauma, CDP-choline accelerated the recovery from post-traumatic coma and the recuperation of walking ability, achieved a better final functional result and reduced the hospital stay of these patients, in addition to improving the cognitive and memory disturbances which are observed after a head trauma of lesser severity and which constitute the disorder known as postconcussion syndrome. In the treatment of patients with acute cerebral vascular disease of the ischemic type, CDP-choline accelerated the recovery of consciousness and motor deficit, attaining a better final result and facilitating the rehabilitation of these patients. The other important use for CDP-choline is in the treatment of senile cognitive impairment, which is secondary to degenerative diseases (e.g., Alzheimer's disease) and to chronic cerebral vascular disease. In patients with chronic cerebral ischemia, CDP-choline improves scores on cognitive evaluation scales, while in patients with senile dementia of the Alzheimer's type, it slows the disease's evolution. Beneficial neuroendocrine, neuroimmunomodulatory and neurophysiological effects have been described. CDP-choline has also been shown to be effective as co-therapy for Parkinson's disease. No serious side effects have been found in any of the groups of patients treated with CDP-choline, which demonstrates the safety of the treatment.

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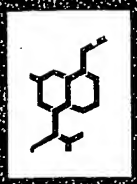
Methods and Findings

in Experimental and Clinical Pharmacology

CDP-Choline: Pharmacological and clinical review



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INTRODUCTION

Phospholipids are essential constituents of cells, more specifically of cell membranes, and they have a very high replacement rate, which implies a continuous synthesis of these compounds to guarantee the smooth functioning of the cell membranes and cells (1-3).

The chemical structure of a phospholipid shows the esterification of a polyalcohol (glycerol or sphingosine) with two long-chain fatty acids and one molecule of phosphoric acid which, in turn, is esterified with nitrogenized bases (choline, ethanolamine), amino acids (serine) or inositol (3, 4). The principal phospholipids in humans are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and sphingomyelin (4). Their main function consists of forming part of the cell membrane structures because they are necessary for membrane functions such as the maintenance of homeostasis and cell compartmentalization, enzymatic activities associated with membrane systems, and the linking between receptors and intracellular signals (1). In the case of the neuronal membrane, they are also involved in the specific functions of conduction and transmission of nerve impulses (1, 5).

Many situations exist in which there is a loss of phospholipids or a reduction in their synthesis, which brings with it an impairment in cell functions and can have physiopathological implications (1).

In the central nervous system, structural phospholipids in the neuronal membrane are necessary for proper cerebral maturation (6-8). In the same way, alterations in the cell membrane and phospholipid metabolism have been implicated in the physiopathology of cerebral edema and traumatic brain injury (9-17), as well as in that of hypoxia (18, 19) and cerebral ischemia (20-28). In addition, it has been found that certain alterations in the neuronal membrane and structural phospholipid metabolism are associated with brain aging (29-31) and with certain neurodegenerative diseases, such as senile dementia of the Alzheimer's type (33-43), as well as with other processes in which changes in neurotransmission (44, 45) and excitotoxic aggression (46, 47) are also involved.

Given these physiopathological conditions, a drug is needed which could, in such situations, accelerate and/or increase the synthesis of membranal structural phospholipids.

Cytidine diphosphate choline (CDP-choline) is a mononucleotide composed of ribose, cytosine, pyrophosphate and choline, whose chemical structure (Fig. 1) corresponds to 2-oxy-4-aminopyrimidine (48). CDP-choline is a necessary intermediate in the synthesis of structural phospholipids in the cell membrane (4, 49-54), since the formation of this compound, starting with phosphorylcholine, is the limiting step in this biosynthetic pathway (52, 55, 56). As shown in Figure 2, CDP-choline is also related to acetylcholine metabolism; in this way, the administration of CDP-choline implies a source of exogenous choline for the synthesis of acetylcholine, as we will discuss later in this article.

PHARMACOLOGICAL ACTIVITIES

Traumatic injury and experimental cerebral edema

Horrocks and Dorman (57) have demonstrated that CDP-choline and CDP-ethanolamine prevent the degradation of choline and ethanolamine phospholipids during decapitation-induced ischemia in rats, and that they produce a partial reversion of the release of free fatty acids during reperfusion after experimental global ischemia in gerbils. Citicoline and CDP-ethanolamine, administered jointly, have a synergistic effect, stimulating the resyn-

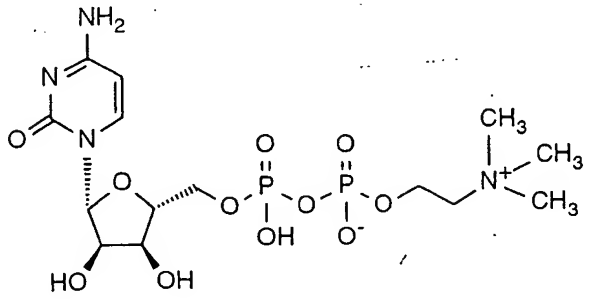

FIG. 1. Chemical structure of CDP-choline.

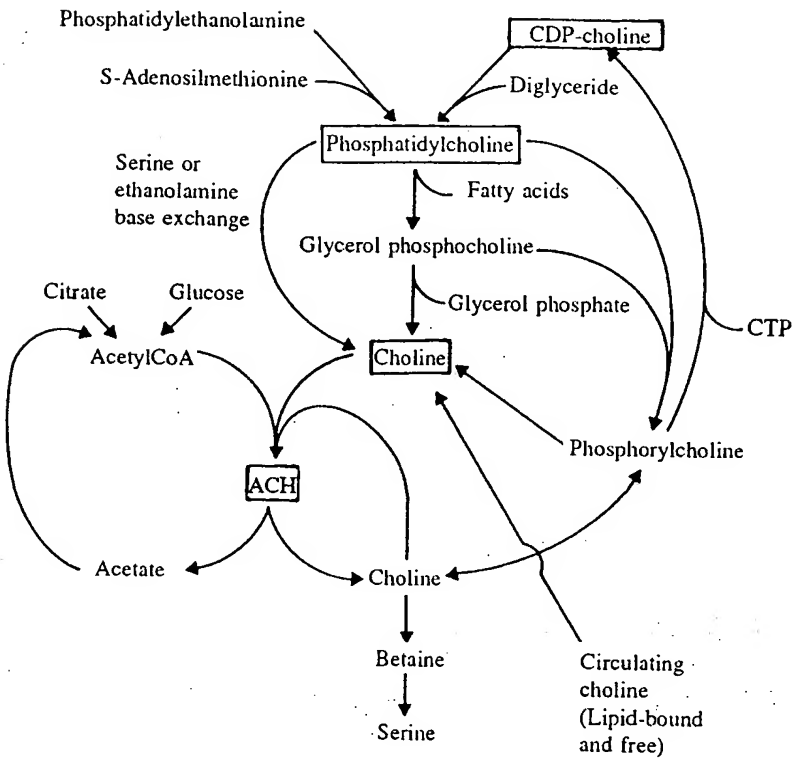

FIG. 2. Relationship between CDP-choline and the metabolism of choline, cerebral phospholipids and acetylcholine.

thesis of choline, ethanolamine and inositol phospholipids, and appreciably reducing the levels of free arachidonic acid.

In an experimental model of acute, induced ischemia in rats, LePoncin-Lafitte *et al.* (58) assessed cerebral edema and the integrity of the brain-blood barrier (using marked iodine albumin) and examined brain metabolism (in histoenzymological studies). By administering citicoline in this experimental model, vasogenic cerebral edema was reduced and the integrity of the blood-brain barrier reestablished. They also demonstrated that with CDP-choline, induced infarcts were smaller and the activities of lactate dehydrogenase, succinic dehydrogenase, monoamine oxidase and acid phosphatase were reduced, emphasizing this drug's protective role through its direct action on the cell membrane.

Mykita *et al.* (59) demonstrated that, in neuronal cultures, the addition of citicoline after a hippocampal lesion resulted in a protection of these cultures. The hippocampus increased incorporation of marked choline in phospholipids, whereas this process was blocked in the presence of citicoline. These authors concluded that CDP-choline was capable of protecting neurons in conditions of alkalosis and of promoting cell proliferation.

In an electrophysiological study in rabbits, Yasuhara *et al.* (60, 61) demonstrated that citicoline comparably reduced wake-up response and muscular exertion thresholds, concluding that it is a useful drug for the treatment of brain injury, due to its effects on consciousness and on the motor activity of the pyramidal system and its afferent pathways.

Martí Viaño *et al.* (62) compared the effects of pyriglutin, piracetam, centrophenoxine and citicoline in a study on the antagonism of barbituric coma in mice. No differences were observed in the animals treated with pyriglutin, piracetam or cethrophenoxine as compared to the control group, whereas with citicoline, both the duration and the depth of the coma were lesser, as was the respiratory depression, in

comparison with the other groups. The alerting effects of citicoline were shown to be due to an increase in cerebral blood flow (CBF), an improvement in the cerebral uptake of O_2 and use of the energy metabolism, and the facilitation of mitochondrial respiration.

In an experimental model of cranial trauma in monkeys, Ogashiwa *et al.* (63) established a significant dose-effect relationship between CDP-choline dosage and coma duration; this relationship was significant from a dose of 60 mg/kg ($p < 0.05$).

Studying the effects of various brain metabolism activators, Watanabe *et al.* (64) found that citicoline increased glucose incorporation and metabolism, decreased lactate accumulation in the brain, and slightly increased cerebral blood flow.

In a study on nerve tissue response to contusion, Alberghina and Giuffrida (9) demonstrated that there was a moderate increase in choline phosphotransferase, accompanied by a greater increase in phospholipase A_2 and in various lysosomal hydrolases. Furthermore, they showed that there was an increase in the number and size of the lysosomes during neuronal regeneration. Arrigoni *et al.* (65) demonstrated that citicoline was capable of completely inhibiting the activation of phospholipase A_2 without changing the activity of choline phosphotransferase. In addition, Freysz *et al.* (66) showed that, in addition to reducing phospholipase A_1 and A_2 activity, CDP-choline reduced the release of free fatty acids in conditions of hypoxia, adding a protective effect to its activating effect on phospholipid reconstruction. Massarelli *et al.* (67) also demonstrated the action of citicoline on phospholipase A_1, which supports the conclusions of other authors. Similarly, Kitazaki *et al.* (68) showed the inhibiting effect of CDP-choline on phospholipase A_2 associated with the cerebral cortex membrane in rats.

Algate *et al.* (69) studied the effects of CDP-choline in an experimental model of epidural compression in anesthetized cats. They observed that the animals treated with citicoline showed greater resistance to the effects of mechanical cerebral compression than the animals of the control group, with the EEG alterations appearing at higher levels of compression. They also observed that in the treated animals, the respiratory and cardiovascular alterations were less intense, concluding that CDP-choline provides significant protection against lethality from epidural compression. These results were comparable to those of Hayaishi (70) and Kondo (71), who found an improvement in the EEGs, as well as in the quality of survival, after the administration of citicoline to cats subjected to experimental cerebral compression.

Tsuchida *et al.* (72) administered [^{3}H]CDP-choline intraperitoneally to rats subjected to dry ice-induced cortical cryogenic lesion, and they confirmed the presence of the marked drug in the cerebral parenchyma, especially in the white matter, and above all in the lesioned areas.

Boismare (10, 73) carried out an experimental model of craniocervical injury without direct blow ("whiplash") in order to assess effects on the levels of central catecholamines; it was found that after the injury, there was an increase in dopamine and a decrease in noradrenaline levels in the brain. This type of lesion produces a postural dysregulation in cerebral blood flow and disturbances in behavior and learning that are related to an acceleration in the degradation of noradrenaline in the brain. In the animals treated with citicoline, the injury did not modify the levels of these amines. The author emphasized the protective role of CDP-choline because of its stabilizing effect on cerebral catecholamine levels.

Clendenon *et al.* (74) found that the decrease in synaptosomal and mitochondrial membranal Mg^{++}-dependent ATPase activity, which is produced in traumatic injury, was prevented with the administration of CDP-choline.

In a series of studies using a model of cryogenic cerebral edema in rabbits, Cohadon *et al.*

(12, 13, 75) showed that with a treatment of 20 mg/kg/day of citicoline, it was possible to:

- Slow the drop in the enzymatic activity of mitochondrial ATPase.
- Restore Na^+/K^+ ATPase activity (Fig. 3).
- Restore the activity of oligomycin-sensitive ATPase.
- Accelerate the reabsorption of cerebral edema, reaching normal values on the fourth day, whereas in the case of spontaneous reabsorption, these levels were not reached until the tenth day.

These authors attributed citicoline's beneficial effects on cerebral edema to two activities: recuperation of membranal enzyme insertion and facilitation of their activity, and reduction of aqueous imbibition by the cerebral parenchyma.

Lafuente and Cervós Navarro (76, 77) carried out a microgravimetric study on ultraviolet radiation-induced cerebral edema in cats in order to evaluate the effect of citicoline on this phenomenon. They divided the animals into 3 groups: the first received citicoline at a dosage of 20 mg/kg, the second at a dosage of 100 mg/kg, and the third served as the control group. After 48 hours, they took a coronal section of the necrotic area and divided it into 6 levels of depth, with level I corresponding to the cortex and level VI to the periventricular area. Determining the level of edema at each level using microgravimetry, they found a reduction in edema, as compared to the control group, in levels I to V with the highest dosage, although there was a paradoxical increase in edema in level VI in all groups. These results indicate that citicoline has a reductional action on the quantity of edema, facilitating the reabsorption of liquid and accelerating its drainage towards the ventricles, i.e., increasing cerebral compliance. The authors concluded that CDP-amines are useful for controlling tissue lesions related to the increase in free fatty acids and for restoring the cell energy metabolism by restarting the Na^+/K^+ pump.

Majem *et al.* (78) assessed the EEG changes that appeared in rats with induced cryogenic edema and how these changes in EEG were modified with the administration of CDP-choline. They observed a significant increase in theta frequency bands during vigilance in treated animals, with a decline in delta and slow alpha waves, and with less intersubject dispersion of the frequency bands on the whole, which was translated into better cerebral electrogenic stability. They concluded that citicoline offers protection against the effects of cryogenic cerebral edema.

In an experimental model of cryogenic cerebral edema, Roda (79) measured the ex-

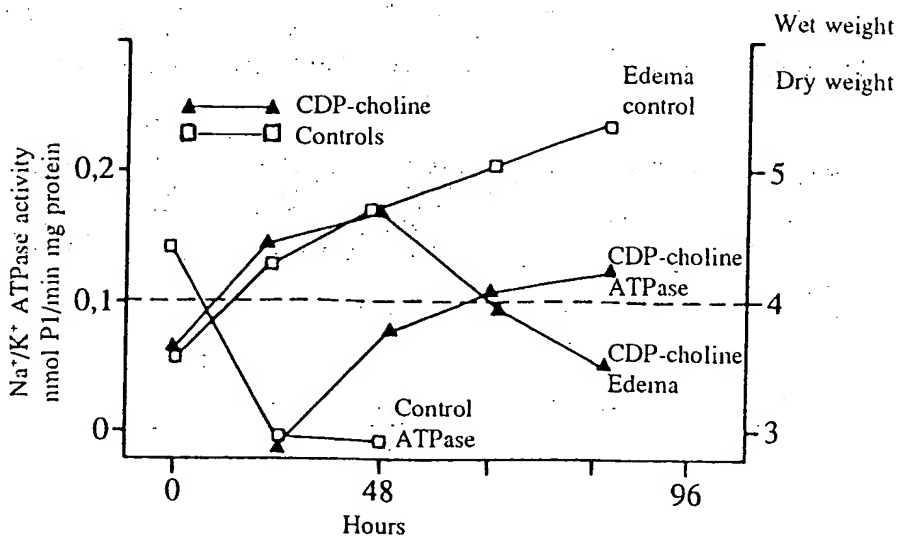

FIG. 3. Evolution of Na^+/K^+ ATPase activity with respect to time and water quantity in cerebral edema.

travasation of Evan's blue across the blood-brain barrier and the uptake of fluorescein by astrocytes and neurons, finding that with the administration of CDP-choline, both processes declined significantly compared to controls, affirming that citicoline has a direct effect on the transmembranal transport of sodium, potassium, water and proteins, in both endothelial cells of the blood-brain barrier and in astrocytes and neurons. Although the exact mechanism of this action is not entirely known, it seems that its effect occurs in the interphase that separates the capillaries from the neuroglia and in the cell membranes.

Due to its biochemical, pharmacological and pharmacokinetic characteristics, CDP-choline is a potentially useful drug for the treatment of traumatic brain injury (80).

Hypoxia and cerebral ischemia

In *in vitro* studies using nerve tissue, it has been shown that anoxia induces a time-dependent drop in the synthesis of structural phospholipids, i.e., the longer the anoxia lasts, the more intense the repercussion on neuronal phospholipid metabolism (81). Furthermore, it has been found that there is a decrease in the incorporation of marked precursors in the phospholipids of neuronal subcellular fractions obtained from animals subjected to experimental hypoxia (18). It is also known that when cerebral ischemia is induced experimentally, the glycerophopholipids of the cell membranes are decomposed by the action of various phospholipids, producing free fatty acids and arachidonic acid derivatives. With the prolongation of ischemia, the aggression induced on these membranes intensifies and they stop functioning, resulting in an accumulation of Na^+ and Ca^{2+} in the interior of the cell, which unfailingly leads to cell death (25, 82).

In conditions of ischemia, with the consequent neuronal damage, endogenous synthesis of CDP-choline is compromised because under these conditions the cell lacks the high energy phosphate compounds necessary for this biosynthetic pathway (83).

Given the importance of restoring neuronal activity after cerebral ischemia (4), and based on the experimental data, many authors have investigated the effects of administering CDP-choline in various experimental models of ischemia and/or cerebral hypoxia.

Boismare *et al.* (84) found that treatment with 20 mg/kg, i.p., of CDP-choline during acute hypoxia induced a reduction in vegetative response, a protection of conditioned avoidance response and a stabilization in the cerebral levels of dopamine and noradrenaline in rats. This same team (85) discovered that dogs subjected to normobaric hypoxia underwent an increase in arterial pressure, heart rate, cardiac output and regional blood flow, without changes in total peripheral resistance. The administration of CDP-choline abolished these hemodynamic effects induced by acute hypoxia, suggesting that this action is correlated with a dopaminergic agonist effect of the drug. In cats subjected to brief periods of cerebral ischemia, these authors (86) observed that a depression in the cortical evoked potentials was produced, a depression which was lessened with the previous intracarotid administration of CDP-choline. These authors consider the protective effects of CDP-choline to be more metabolic than hemodynamic in origin, and they do not rule out the possibility of the drug producing a direct effect on central dopaminergic structures.

Alberghina *et al.* (87) investigated the effect of CDP-choline on the incorporation of marked precursors in the cerebral phospholipids of guinea pigs subjected to hypoxia. One group of animals received 100 mg/kg CDP-choline intraperitoneally, and 10 minutes later the marked precursors [2-^{3}H]glycerol and [1-^{14}C]-palmitate were administered intraventricularly. Another group of animals, the control group, only received the precursors. The researchers observed that, in comparison with the control group, the animals treated with CDP-choline showed an increase in total lipid and phospholipid specific radioactivity in purified mitochondria obtained from the brain hemispheres,

the cerebellum and the brain stem. In a subsequent study, this same group of researchers (88) found that CDP-choline was capable of reversing the effects of hypoxia on the incorporation of marked precursors in RNA and proteins, especially in the nucleus and mitochondria.

In many experimental studies, it has been demonstrated that CDP-choline prevents the release of fatty acids during ischemia and cerebral hypoxia and increases the synthesis of structural phospholipids, (89-95). Using an experimental model of decapitation-induced global cerebral ischemia, Horrocks *et al.* (89, 92, 94) showed that the administration of a combination of CDP-choline and CDP-ethanolamine reduced the release of free fatty acids and increased the synthesis of corresponding glycerophospholipids, suggesting the participation of choline and ethanolamine phosphotransferases. Using an experimental model of bilateral carotid ligature-induced global ischemia in gerbils, Trovarelli *et al.* (90, 91) found that the intraperitoneal administration of CDP-choline partially prevented the changes in lipid metabolism caused by cerebral ischemia, correcting the increase in free fatty acids, the changes in neutral lipids such as diacylglycerol and the decrease in phosphatidylcholine. Suno and Nagaoka (93) studied the effects of the administration of CDP-choline on the release of free fatty acids produced by a 5-min complete cerebral ischemia in rats. They found that the drug in question reduced the increase in free fatty acids; the intensity of this effect depended on the dosage used. The arachidonic acid content in the brains of the control animals subjected to ischemia was 174±22 mmol/g, whereas in the animals that received 200 and 1000 mg/kg CDP-choline, i.p., it was 119±8 mmol/g and 61±8 mmol/g, respectively (Fig. 4). The authors concluded that these results indicated that the administration of CDP-choline can prevent cerebral ischemic damage. Agut and Ortiz (95) treated male rats weighing 190-200 g with 4 mg/kg of oral [^{14}C]methyl CDP-choline

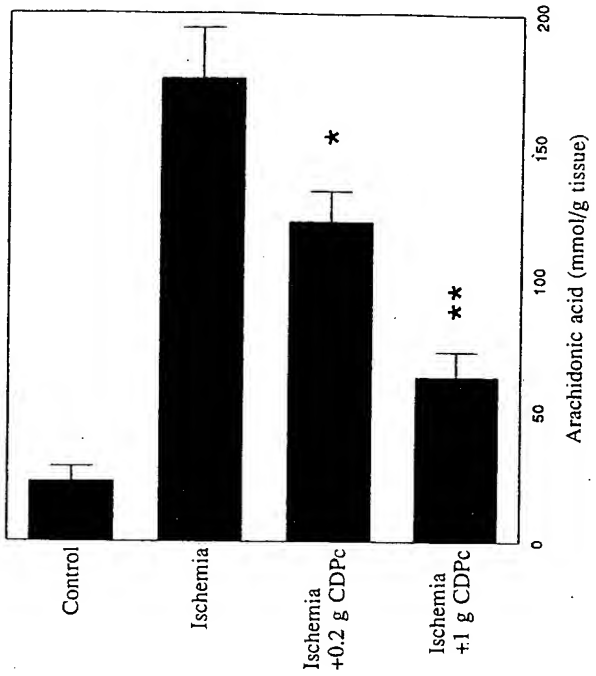

FIG. 4. Effect of CDP-choline on arachidonic acid released in ischemic rat brain. CDP-choline (200 and 1000 mg, i.p.) was administered 10 min before decapitation, and 5 min later free fatty acids were extracted. Arachidonic acid was determined by gaseous chromatography. *p <0.05; **p <0.001 as compared to ischemia without treatment. CDPc: CDP-choline.

(50 μm Ci). After 24 hours, the cerebral radioactivity level and the presence of marked phospholipids were determined in conditions of normoxia, hypoxia and hyperoxia after the additional administration of 100 mg/kg of unmarked CDP-choline. The researchers found an accentuated incorporation of radioactivity in the brains of normoxic and hypoxic animals, for the most part associated with phosphatidylcholine. Furthermore, the administration of unmarked CDP-choline reduced the elevation of cerebral lysophosphatidylcholine caused by hypoxia. Tornos *et al.* (96) carried out a pharmacological study on the protective effect of CDP-choline on toxicity in an experimental model of potassium cyanide-induced hypoxia and found that oral treatment with CDP-choline for 4 days previous to the induction of hypoxia had a protective effect that was manifested in a longer survival time in treated animals.

These beneficial effects of CDP-choline can also be ascribed to the activation of the cerebral energy metabolism (97) and to the increase in the activity of mitochondrial cytochrome oxidase (98) induced by this drug.

Narumi and Nagaoka (99) investigated the effect of CDP-choline administration on cerebral monoamine metabolism in two models of global cerebral ischemia in rats. In the first model, they induced a 30-min cerebral ischemia with bilateral carotid occlusion in spontaneously hypertensive rats, observing an important decline in noradrenaline levels in the cerebral cortex. In this model, the administration of 1000 mg/kg CDP-choline reduced the dopamine content in the striatum and diencephalon, normalizing the ischemia-induced decrease in the dopamine/dopamine metabolite ratio. In the second model, a bilateral carotid occlusion was produced 24 hours after the electrocauterization of both vertebral arteries in Wistar rats. In this model, it was observed that the levels of noradrenaline, dopamine and serotonin declined 70-80% in the cerebral cortex. Similar decreases were also observed for noradrenaline and serotonin in the hippocampus, for dopamine in the nucleus acumbens, for dopamine and serotonin in the striatum and for noradrenaline in the diencephalon and brain stem. The administration of CDP-choline at a dose of 500 mg/kg significantly increased the ischemia-induced drop in dopamine levels in the striatum, which led the authors to suggest that CDP-choline may cause a replacement of striatal dopamine in rats subjected to experimental cerebral ischemia.

Nagai and Nagaoka (100) published the results of a study in which they investigated the effect of CDP-choline on glucose uptake in various areas of the brain of rats that had undergone global cerebral ischemia induced by 30-min occlusion of both carotid arteries, after having electrocauterized both vertebral arteries. After four days of recirculation, they proceeded to determine cerebral glucose uptake. They found that without the administration of CDP-choline, global cerebral uptake was reduced to 81% of the normal value. With the administration of CDP-choline at a dose of 250 mg/kg, i.p., twice a day for 3 days after the start of recirculation, the postischemic reduction in glucose uptake was significantly less in the cerebral cortex (Table 1). This suggests that CDP-choline improves cerebral energy metabolism in conditions of ischemia.

Kakihana *et al.* (101) investigated the distribution of marked CDP-choline and its effects on acetylcholine synthesis from glucose in the cerebral cortex of rats subjected to 30-min ischemia followed by reperfusion. The rats were sacrificed by microwave irradiation 3 and 60 minutes after the administration of marked CDP-choline, which was administered 10 min before initiating reperfusion. The quantity of radioactivity collected per gram of tissue in the soluble acid fraction was 0.2 and 0.07% of the

TABLE 1. Effect of CDP-choline on glucose uptake in several cerebral regions in rats subjected to experimental cerebral ischemia. All values represent mean ± SD for 7 rats.

	Glucose uptake (mg/g/10 min)		
		Rats subjected to cerebral ischemia	
Region	Normal rats	Saline	CDP-choline
Frontal cortex	3.317±0.106	2.546±0.144**	2.931±0.090#
Parietal cortex	3.250±0.114	2.232±0.145**	2.652±0.031#
Occipital cortex	3.250±0.118	2.175±0.160**	2.563±0.105#
Temporal cortex	2.671±0.042	2.106±0.106**	2.410±0.041##
Nucleus striatum	2.703±0.074	2.152±0.192*	2.249±0.053
Thalamus	3.130±0.132	2.421±0.141**	2.655±0.139

*p <0.05; **p <0.01 compared to normal rats.

#p <0.05; **p <0.01 compared to rats subjected to ischemia and treated with saline solution.

dose injected after 3 and 60 min, respectively. Analysis with high performance liquid chromatography showed that 1 and 6% of the radioactivity of the soluble acid fraction was collected in the acetylcholine fraction at 3 and 60 min, respectively. Another group of animals was administered 250 mg/kg CDP-choline, i.p., twice a day for 3 days after ischemia-reperfusion. On the fourth day, marked glucose was administered intravenously, 30 min after the last administration of CDP-choline. The rats were sacrificed 3 min after completing glucose administration. In the control group, which had been treated with saline solution, it was observed that ischemia tended to cause an increase in glucose and pyruvate levels in the cerebral cortex, accompanied by a 50% decline in the synthesis of acetylcholine from glucose. Treatment with CDP-choline improved the metabolism of glucose and significantly restored the synthesis of acetylcholine from glucose. For these authors, the results obtained indicate that CDP-choline improves cerebral energy metabolism in conditions of ischemia. Subsequently, these same authors (102) assessed the effects of CDP-choline on neurological sequelae and cerebral glucose metabolism in an experimental model of transitory cerebral ischemia in rats, demonstrating that CDP-choline at high doses improved the neurological state of the animals subjected to ischemia (Figs. 5 and 6), which was correlated with an improvement in the cerebral energy metabolism and with the incorporation of the drug in the membrane phospholipid fraction. These results coincide with those obtained by Fukuda *et al.* (190) in a preliminary study.

Nagaoka (104) studied the effects of CDP-choline on the initiaiton of stroke and mortality in spontaneously hypertensive rats subjected to cerebral ischemia. Ischemia was induced by the occlusion of both common carotid arteries. CDP-choline (200-1000 mg/kg, i.p.), administered previous to the induction of ischemia, caused a dose-dependent delay in the initiation of stroke and respiratory arrest; these effects were also observed in animals treated after the induction of ischemia. Furthermore, CDP-choline, at a dose of 500 mg/kg, i.p., improved the neurological state of rats subjected to 40 min of cerebral ischemia followed by reperfusion. These results suggest that CDP-choline exercises a neuroprotective role against cerebral ischemia and reperfusion.

Saligaut and Boismare (105) studied the effects of CDP-choline, administered at a dose of 1000 mg/kg, p.o., in Wistar rats subjected to acute hypobaric hypoxia (15 min at a simulated altitude of 7180 meters), assessing the results of a behavioral conditioning test and the uptake of dopamine in the striatum, as well as levels of this neurotransmitter and its metabolites in the striatum. In the behavioral conditioning test it was observed that CDP-choline protects against hypobaric hypoxia in a manner different from and superior to apomorphine. In the biochemical studies, a presynaptic effect was shown, probably due to the activation of tyrosine hydroxylase, which induced changes in dopamine uptake, as well as an improvement in its release.

Le Poncin-Lafitte *et al.* (58) studied the effects of CDP-choline on various brain tissue alterations in an experimental model of multifocal cerebral ischemia in cats, in which the ischemic lesion was produced by the introduction of calibrated microspheres into the internal carotid artery, producing cerebral microinfarcts characterized by their central zone of necrosis surrounded by a zone of penumbra, together with edema caused by breaking the blood-brain barrier. With the administration of CDP-choline, the number of lesions diminished considerably, as did the quantity of albumin extravasated, which confirms, for these authors, CDP-choline's neuroprotective role against ischemia due to its activity on the cell membranes.

Araki *et al.* (106) also found CDP-choline to have a certain neuroprotective effect in complete cerebral ischemia induced by decapitation and potassium cyanide intoxication in mice.

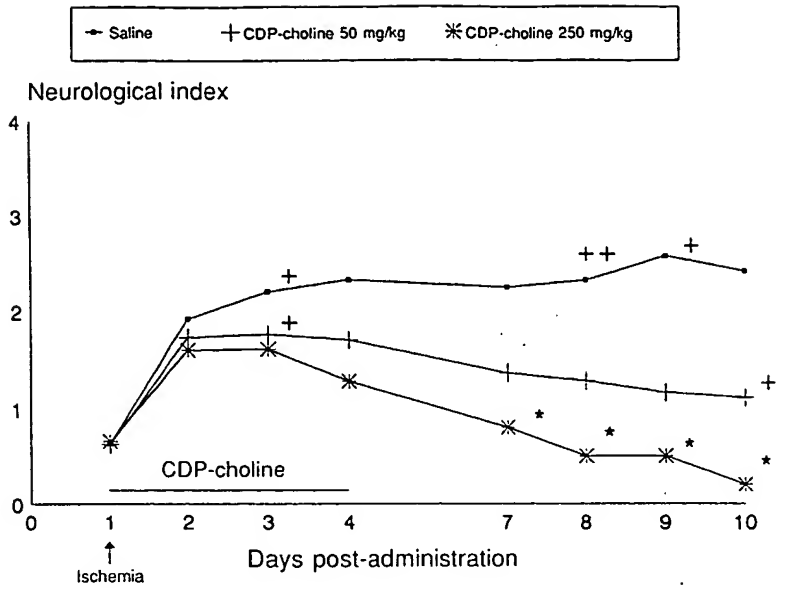

FIG. 5. Effect of CDP-choline (50 and 250 mg/kg, i.p., twice daily for 4 days) on changes in the neurological index for 10 days following 20-min cerebral ischemia in rats (n=10). *p =0.05 *vs.* saline solution; +: a dead rat.

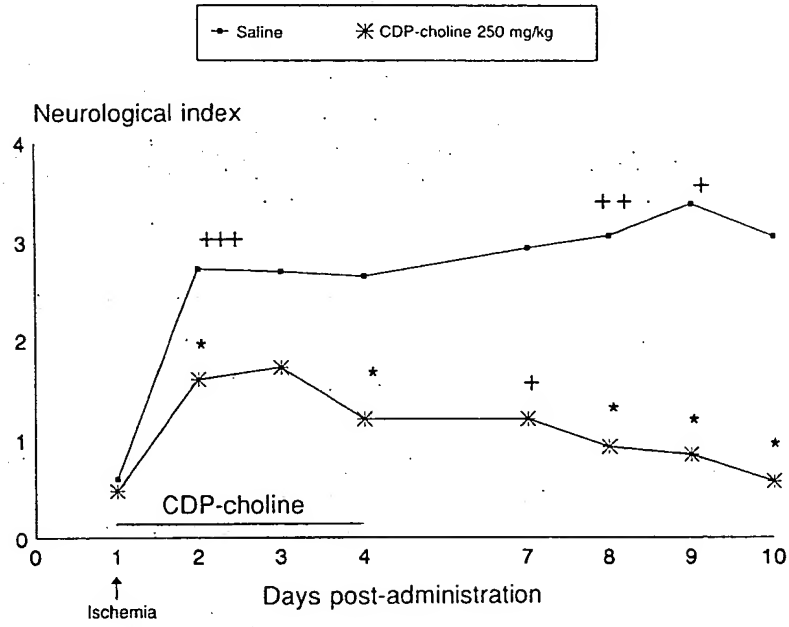

FIG. 6. Effect of CDP-choline (250 mg/kg, i.p., twice daily for 4 days) on changes in the neurological index for 10 days following 30-min cerebral ischemia in rats (n=10). *p =0.05 *vs.* saline solution; +: a dead rat.

Hamdorf and Cervós-Navarro (107) kept 48 rats exposed to chronic hypoxia with a decreasing quantity of oxygen for 103 days. For each of the levels of hypoxia (15, 12, 10, 8 and 7% of inhaled oxygen), the behavior of the animals in an open field was determined. Twenty-four of the animals received CDP-choline, administered with the food, at a dose of 100 mg/kg. During hypoxia, reactions indicative of impaired alertness were noted. CDP-choline showed a protective effect by increasing alertness in conditions of moderate hypoxia (15% O_2). In a later study, these same authors (108) analyzed the effects of CDP-choline in Wistar

rats subjected to hypoxia for 5 months. Eighty rats were maintained in a gradually hypoxic environment, establishing levels of hypoxia on the order of 15, 12, 10 and 7% O_2 in inhaled air in order to subsequently record their behavior in an open field. One group of 40 animals received CDP-choline at mealtimes, at a dose of 100 mg/kg. The behavioral changes induced by hypoxia were mitigated in the group of animals treated with CDP-choline. It is important to note that the therapeutic administration of CDP-choline was more effective than its prophylactic administration. Furthermore, in conditions of extreme hypoxia, CDP-choline was demonstrated to have a protective effect by prolonging survival.

In addition, Masi *et al.* (109) have shown that CDP-choline has a certain degree of platelet anti-aggregation effect, which could imply an additional benefit in the treatment of cerebral vascular disease. These authors analyzed the effects of acute (250 mg/kg) and chronic (250 mg/kg/day for 2 weeks) administration of CDP-choline on platelet aggregation, on the formation of thromboxane and on platelet anti-aggregation activity in the thoracic aorta in rats. Acute administration produced, principally, a reduction in the platelet reactivity of aggregation agents, but no changes in thromboxane production were noted. After chronic treatment, the main effect observed was an increase in the anti-aggregation activity of the vascular wall, although the platelet function was not altered. Consequently, CDP-choline produces favorable effects which lead to the reduction of platelet reactivity, especially in acute treatment.

According to Drago *et al.* (110), CDP-choline is a drug of choice for the treatment of cerebral vascular disease, especially when given chronically, since its clinical use is justified by the pharmacological actions it has on the central nervous system. CDP-choline stimulates dopamine synthesis in nigrostriatal areas and antagonizes the alterations in dopamine and noradrenaline concentrations caused by various noxa in the central nervous system. It also stimulates cholinergic neurotransmission. In various models of cerebropathy, CDP-choline has improved or normalized functional and biochemical parameters of the central nervous system. It stimulates the synthesis and inhibits the catabolism of cerebral phospholipids and exerts a protective effect on membranal ATP-ase and on enzymes involved in the cerebral energy metabolism, especially succinic dehydrogenase and citrate synthetase, as well as on the metabolism of proteins and nucleic acids, increasing the biosynthesis of RNA in certain regions of the brain. In models of chronic cerebral ischemia, CDP-choline improves neurological deficit and prolongs survival. In addition, it favors neuronal synaptogenesis and effects an inhibitory action on the activity of certain phospholipases. Therefore, CDP-choline has three principal mechanisms of action which are valid for the treatment of cerebral vascular disease:

- Interference with cerebral energy metabolism.
- Stimulation of central neurotransmission.
- Activation of cell repair mechanisms.

Synaptic transmission and neurotransmitter levels

As previously mentioned, CDP-choline produces some of its effects by acting on the levels of certain neurotransmitters. In this section we are going to analyze concretely which effects it has on neurotransmission. As will be explained later, most studies have focused on the analysis of CDP-choline's effect on central dopaminergic transmission.

Martinet *et al.* (111) carried out a study in rats in which they determined the effects of CDP-choline administration on noradrenaline, dopamine and serotonin levels in different regions of the brain. To do this, they determined the conversion of intravenous [^{3}H]tyrosine and [^{3}H]tryptophan to [^{3}H]noradrenaline, [^{3}H]dopamine and [^{3}H]serotonin, comparing the results obtained with the administration of

saline to those obtained after administering CDP-choline at various doses. The metabolism of each neurotransmitter was studied in the regions of the brain in which it is functionally active. Thus, the activity of CDP-choline was studied in the corpus striatum, cerebral cortex and mesencephalon for catecholamines, while for serotonin, it was also studied in the hypothalamus. The speed of dopamine, noradrenaline and serotonin synthesis was expressed as a conversion index equal to the quotient of the quantity of marked neurotransmitter per gram of brain (cpm/g) and the specific radioactivity of tyrosine or tryptophan in the brain. As shown in Figure 7, CDP-choline significantly increased the level and speed of dopamine synthesis in the corpus striatum, with similar effects on tyrosine levels. Noradrenaline levels were increased in the cortex, but no changes in the brain stem were noted with respect to the controls. In terms of the effects on serotonin, the drug produced a decrease in the levels and speed of synthesis of this neurotransmitter in the brain stem and hypothalamus, with no alterations observed in the cortex or striatum. According to these authors, the increase in dopamine synthesis could be attributed to an enhancing effect of CDP-choline on tyrosine hydroxylase, which constitutes the limiting step in dopamine synthesis. This activation of tyrosine hydroxylase is accompanied by an inhibition of the re-uptake of dopamine in the synap-

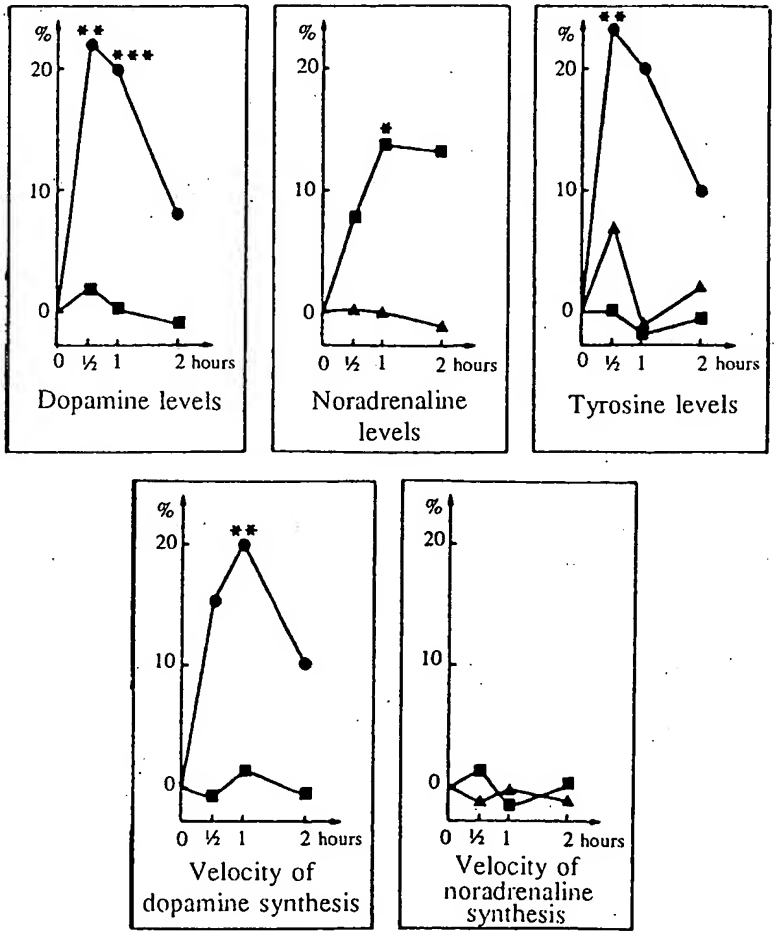

FIG. 7. Influence of CDP-choline (30 mg/kg, i.v.) on catecholamine synthesis at different times after its administration. Graphs represent variations in catecholamine levels and synthesis velocity, in percentages as compared to the control and in different localizations (● corpus striatum; ■ cortex; ▲ cerebromesencephalic stem). *p <0.1; **p <0.05; ***p <0.01.

ses, an activity which has been demonstrated in *ex vivo* studies (112, 113). It does not seem, however, that the observed increase in dopamine synthesis is related to the increase in tyrosine levels, since tyrosine completely saturates tyrosine hydroxylase in physiological conditions. The effects of CDP-choline on dopamine synthesis in the striatum are of special interest if we keep in mind that changes in the synthesis of this neurotransmitter by extrapyramidal dopaminergic neurons are found in the origin of Parkinson's disease.

Saligaut *et al.* (114) obtained results consistent with those previously mentioned with respect to dopamine re-uptake in synaptosomes obtained from the striata of rats pretreated with CDP-choline. After chronic treatment with this drug, there was an observable decline in the synaptosomal re-uptake of dopamine, and the authors attributed this fact to the increase in tyrosine hydroxylase activity, which would be accompanied by an increase in dopamine synthesis. They also consider that a structural modification in the neuronal membranes, fundamentally at the level of phospholipids, could be one of the causative factors in the CDP-choline-induced change in synaptosomal re-uptake of this neurotransmitter. They observed that hypobaric hypoxia antagonized the inhibitory effect of CDP-choline on the synaptosomal re-uptake of dopamine. This antagonism can be explained by the fact that hypoxia reduces the activity of tyrosine hydroxylase, an enzyme which requires oxygen, counteracting CDP-choline's activation of this hydroxylase with a decrease in dopamine synthesis and resulting increase in re-uptake. These same authors studied CDP-choline's activity on oxotremorine-induced cholinergic symptoms in mice (115), demonstrating that pretreatment with CDP-choline did not enhance these symptoms, but did inhibit oxotremorine-induced salivation. Levodopa antagonizes trembling-akinesia type cerebral symptoms induced by oxotremorine; however, this antagonism disappears in animals treated chronically with oral CDP-choline, an observation which confirms its action on dopaminergic pathways. The effects of CDP-choline seem to be mediated by the hypersensitivity of some dopaminergic receptors and not by direct stimulation of striatal dopaminergic receptors. In another series of experiments, these authors studied the effects of CDP-choline on catecholamine metabolism in the striatum and hypothalamus of rats subjected to acute hypobaric hypoxia (116). The hypoxia induced a decline in the levels of homovanillic acid, dihydroxyphenylacetic acid and 3-methoxytyramine in the striatum, and of noradrenaline in the hypothalamus, while it increased dopamine levels in both localizations. The administration of CDP-choline at an oral dosage of 1000 mg/kg partially reversed the effects of hypoxia on 3-methoxytyramine, dopamine and noradrenaline, while it enhanced these effects on homovanillic acid and dihydroxyphenylacetic acid. These results show that CDP-choline partially offsets the effects of hypoxia on the release and metabolism of certain neurotransmitters. In another study, Saligaut *et al.* analyzed the effects of CDP-choline on rats with 6-hydroxydopamine-induced unilateral lesion in the in the nigro-striatum (117). In the lesioned animals, the administration of amphetamine induced an ipsiversive circular behavior, and seemed to be mediated by the development of a supersensitivity of the postsynaptic dopaminergic receptors on the lesioned side. Subchronic treatment with CDP-choline did not cause behavioral effects. CDP-choline did not modify the stimulating effect of apomorphine, but it did enhance the effects of levodopa and amphetamine. These data show that the effects of CDP-choline are mediated by a presynaptic mechanism. Although the enhancement of levodopa cannot be explained by the activation of tyrosine hydroxylase, this effect seems to be related to an improvement in the release of dopamine synthesized from exogenous levodopa.

Agut *et al.* (118) indirectly studied CDP-choline's effect on dopamine synthesis in the

striatum by measuring local levels of dopamine metabolites in animals with a haloperidol-induced blockage of dopaminergic receptors. Pretreatment with CDP-choline, 100 mg/kg/day for 5 days, significantly increased homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the striatum of treated animals, as compared to the control group (Table 2). The increase in the levels of these metabolites was even more marked in a group of animals who also received apomorphine. The results of this study indicate that CDP-choline increases dopamine synthesis in the striatum of rats who have undergone a haloperidol-induced activation of dopamine synthesis. This same team of researchers later carried out a study to determine whether CDP-choline, on its own and without the provocation of a greater demand for dopamine on the part of the dopaminergic receptors, caused increased synthesis of this neurotransmitter, which would be reflected in an increase in the levels of its principal metabolites, HVA and DOPAC, in the striatum (119). CDP-choline was administered orally at a dose of 100 mg/kg and 2 g/kg, respectively, to two groups of animals (5 per group) three times consecutively at intervals of one hour. A third group with similar characteristics received 10 ml/kg water, following the same protocol. Thirty minutes after the last administration, the animals were sacrificed, their brains removed, and the levels of HVA and DOPAC in the corpus striatum determined. DOPAC levels in the striatum increased 60% (p <0.05) with the 100-mg/kg dose of CDP-choline, and 140% (p <0.01) with the 2-g/kg dose. HVA levels, however, were not modified by the 100-mg/kg dose, but the higher dose produced an increase of 50% (p <0.01) in striatal levels.

TABLE 2. Effect of CDP-choline on HVA and DOPAC levels (mean±SD) in the striatum of rats that received haloperidol. Groups of 5 rats recevied CDP-choline (100 mg/kg, p.o.) or water for 5 days; on the 5th day the animals received haloperidol (0.15 mg/kg, i.p.) 90 min before sacrifice. HVA and DOPAC levels were determined in the striatum.

	DOPAC (µg/g)	HVA (µg/g)
Water+haloperidol	4.54±1.14	3.84±0.79
CDP-choline+haloperidol	5.59±1.12*	4.76±0.38*

*p <0.01 compared to control.

CDP-choline's activity on the dopaminergic system has also been studied by examining its pharmacological activity in experimental models of apomorphine-induced hypothermia, haloperidol-induced tardive dyskinesia and acrylamide-induced lesion. Agut *et al.* (120) studied the effect of CDP-choline administration on apomorphine-induced hypothermia, which is considered to be a consequence of the agonist action of apomorphine on D_2 receptors. In addition to apomorphine, the experimental animals received haloperidol at a dose sufficient to partially block the hypothermia induced by apomorphine in order to obtain a pharmacological system sensitive to CDP-choline's activity on the dopaminergic system. One group of animals received an oral dose of 100 mg/kg CDP-choline, followed by the administration of 0.15 mg/kg of haloperidol, i.p., 30 min later. After an additional 30 min, rectal temperature was measured and 1 mg/kg of apomorphine was administered subcutaneously. Rectal temperature was subsequently measured at 30, 60 and 90 min. Another group of animals received water instead of CDP-choline, following the same protocol. The effects of chronic CDP-choline, at a dose of 100 mg/kg/day, p.o., for 5 days were also analyzed, following the same protocol on the last day as in the acute administration. The average decreases in temperature observed in each group of animals at the different times of measurement are shown in Table 3. Acute administration of CDP-choline produced hypothermia that was significant at all times of measurement. With chronic administration, a significant result was obtained only at 90 min. The authors concluded that a 100-mg/kg oral dose of CDP-choline under acute

administration produces a hypothermal effect similar to that described for various dopaminergic agonists. In addition, they assert that the fact that chronic administration of CDP-choline produces significant hypothermia only at the last measured time probably reflects that when administered by this route, the drug acts predominantly on the synthesis of phospholipids rather than on the synthesis of acetylcholine. It is this second pathway of action which would predominate with acute administration, since it implies a relatively fast use of the choline delivered, which would be used for the synthesis of acetylcholine, bringing about an increase in cholinergic interneuron-mediated tyrosine hydroxylase activity. On the other hand, the chronic administration of this drug would be accompanied by an increasingly greater availability of cytidine, thus diverting cerebral choline towards CDP-choline and phospholipid synthetic pathways, which would indirectly involve a dopaminergic agonist effect. From a study which included the chronic administration of haloperidol or water to a total of 120 animals, these authors developed an experimental model of haloperidol-induced (2 mg/kg/day for 7 days) tardive dyskinesia in rats (121). After a 7-day interruption in treatment, the animals received water or apomorphine and their motor activity was determined. The animals treated with apomorphine (0.25 mg/kg) showed hypermotility ($p < 0.001$) as compared to the animals who received water, or to those not treated with haloperidol who received the same dose of apomorphine. In the rats treated with haloperidol who received CDP-choline (500 mg/kg/day for 7 days) but not apomorphine, hyperactivity was also observed ($p < 0.001$) in comparison with those treated with haloperidol who received water. The administration of CDP-choline plus apomorphine in rats treated with haloperidol produced motor activity similar to that observed in the group that only received CDP-choline. The data from this study show that in a model of haloperidol-induced dopaminergic hypersensitivity, the oral administration of CDP-choline induced hypermotility; this could cause competition with other agonists, giving rise to a partial reduction in the effect of apomorphine in animals pretreated with CDP-choline. In a model of acrylamide-induced lesion, these same authors (122) showed that the administration of low doses of oral CDP-choline (50 mg/kg) were effective in correcting the neurological syndrome induced by acrylamide. Similarly, the simultaneous administration of both substances, which induced a notable weight loss in mice, has been demonstrated to cause an activation of the dopaminergic system, as was seen in the results of the apomorphine stereotype test.

Using fluorimetry, Shibuya *et al.* (123) measured striatal dopamine levels after the administration of a single 500 mg/kg dose of CDP-choline, i.p., and found that a significant ($p < 0.05$) increase in dopamine was produced in the striatum one hour after injection. In addition, Stanzani (124) showed that CDP-choline has a neuroprotective effect on the substantia nigra by observing how it protects this area against the lesions induced by horseradish peroxidases obtaining a greater number of surviving cells. Porceddu and Concas (125) also described a trophic and/or stimulating effect of CDP-choline on nigrostriatal dopaminergic neurons in a model of kainic acid-induced lesion. Miwa *et al.* (126) suggest that CDP-choline may act as an inhibitor of dopamine re-uptake after administration of a single dose and that this drug may modify dopaminergic neuronal activity by changing the composition of the neuronal membrane through repeat administration. Furthermore, these authors found that CDP-choline has muscarinic effects. Giménez *et al.* (127) showed that the chronic administration of CDP-choline to aged mice promotes a partial recuperation of muscarinic and dopaminergic receptor functioning, which normally declines with aging. In their opinion, this action can be explained by the mechanisms involved in neuronal membrane fluidity, supporting the results obtained by

TABLE 3. Temperature drop for each batch studied, referred to time zero and expressed as mean for n=20.

Batch	Drugs	Time		
		+30 min	+60 min	+90 min
A	Water (10 ml/kg, p.o.) Apomorphine (1 mg/kg, s.c.) Haloperidol (0.15 mg/kg, i.p.)	1.19±0.23	0.61±0.17	0.19±0.15
B	CDP-choline (0.1 g/kg, p.o.) Apomorphine (1 mg/kg, s.c.) Haloperidol (0.15 mg/kg, i.p.)	1.39±0.18**	0.74±0.17*	0.38±0.14**
C	Water (10 ml/kg/5 d, p.o.) Apomorphine (1 mg/kg, s.c.) Haloperidol (0.15 mg/kg, i.p.)	1.13±0.22	0.63±0.25	0.26±0.12
D	CDP-choline (0.1 g/kg/5 d, p.o.) Apomorphine (1 mg/kg, s.c.) Haloperidol (0.15 mg/kg, i.p.)	1.11±0.25	0.70±0.19	0.41±0.12**

*p <0.05; **p <0.01 with respect to control.

Petkov *et al.* (128). Comparing the effects of CDP-choline with those of the nootropes adaphenoxate and meclophenoxate on the cerebral levels of the biogenic monoamines noradrenaline, dopamine and serotonin in the frontal cortex, striatum, hippocampus and hypothalamus in rats, Petkov *et al.* (129) found that adaphenoxate increased noradrenaline levels in the striatum and decreased them in the hypothalamus, increased dopamine levels in the cortex and hypothalamus and decreased them in the striatum, and increased serotonin levels in the cortex and decreased them in the hippocampus. Meclophenoxate induced a decrease in noradrenaline levels in the cortex and hypothalamus, increased dopamine levels in the hippocampus and hypothalamus, and increased serotonin levels in the cortex, striatum, hippocampus and hypothalamus. CDP-choline increased noradrenaline levels in the cortex and hypothalamus, dopamine levels in the striatum, and serotonin levels in the cortex, striatum and hippocampus, presenting a slightly different profile from that of the nootropes. In terms of CDP-choline's activity on noradrenaline, the study by López G.-Coviella *et al.* (130) showed that the administration of CDP-choline increased total urinary excretion of 3-methoxy-4-hydroxyphenilglycol, which reflects noradrenergic activity in rats and humans, suggesting that CDP-choline increases noradrenaline release.

In summary, the effects of CDP-choline have been studied in experimental models which are used to reveal its pharmacological activity on the dopaminergic system. It has been demonstrated that CDP-choline acts as a dopaminergic agonist, with especially significant effects on the striatal levels of dopamine and its metabolites. The results indicate that the administration of CDP-choline increases striatal dopamine synthesis, probably by activating tyrosine hydroxylase. The increase in dopamine levels would be determined, in part, by an inhibition in its re-uptake, which is possibly related to CDP-choline's activity on phospholipid synthesis. This drug also has effects on the other monoamines, serotonin and noradrenaline, and on the muscarinic receptors.

Learning and memory performance and cerebral aging

It has been demonstrated that hypobaric hypoxia diminishes learning capacity in rats subjected to sonic avoidance conditioning and

that it is possible to antagonize this effect using pretreatment with apomorphine or other dopaminergic agonists. These effects of hypoxia appear together with an inhibition of cerebral catecholamine metabolism, which in the long run would be the cause of a hypostimulation of central postsynaptic dopaminergic receptors. Starting with these premises, Saligaut and Boismare (105) carried out a study on the effects of CDP-choline administration on learning capacity in rats subjected to hypobaric hypoxia. CDP-choline, at a dose of 300 mg/kg/day for 12 days, was administered in conditions of hypoxia to a group of rats who, in the last 5 days of treatment, were subjected to learning tests in a sonic avoidance conditioning model. The effects observed in this group were compared with those of another group that received 0.5 mg/kg of apomorphine 30 minutes before each daily conditioning session, and with observations of animals that received both treatments. One group of animals served as controls, receiving a solution of ascorbic acid under the same experimental conditions. In Figure 8, the average percentage of avoidances for each of the groups and for each of the days in which they were subjected to avoidance conditioning are shown, demonstrating that CDP-choline partially restored learning capacity. The same effect, although to a lesser extent, was observed with apomorphine administration and with the co-administration of both drugs. These results indicate that when administered, CDP-choline, like the dopaminergic agonists, counteracts the effects of hypoxia.

Drago *et al.* (131) administered 10-20 mg/kg/day of CDP-choline intraperitoneally for 20 days to 24-month-old male Sprague-Dawley rats belonging to a strain with cognitive and motor deficits. The drug was also administered to rats with behavioral changes induced pharmacologically by a single injection of scopolamine, a cholinergic antagonist, by prenatal exposure to methylazoximethanol, or by bilateral injections of kainic acid in magnocellular basal nuclei. In all cases, CDP-choline improved learning capacity and memory, as assessed by active and passive avoidance tests. In the group of aged rats, an improvement in motor capacity and coordination was also observed. In the opinion of these authors, such results indicate that CDP-choline affects the central mechanisms involved in cognitive behavior, probably by means of cholinergic action.

In a model of scopolamine-induced memory impairment, Petkov *et al.* (132) found that

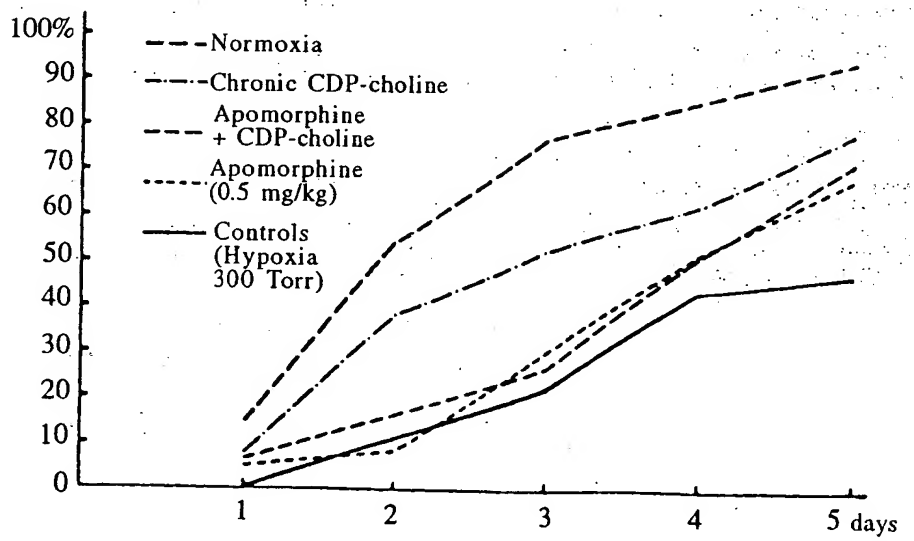

FIG. 8. Effect of CDP-choline and/or apomorphine (0.5 mg/kg) on learning (avoidance) in the hypoxic rat. Performance of normotoxic animals is only indicative.

CDP-choline was able to prevent scopolamine-induced amnesia. Subsequently, Mosharrof and Petkov (133) showed that CDP-choline at a dose of 100 mg/kg completely prevented amnesia induced by scopolamine, and that the combination of 50 mg/kg of CDP-choline with 500 mg/kg of piracetam produced, in addition, a significant increase in retention. The authors suggest that this effect is mediated by the drug's activity on neurotransmission. CDP-choline acts as a memory-enchancing drug, and this effect is especially pronounced in animals with memory deficit (134).

Cerebral aging is characterized by many physiological, neurochemical and morphological changes in mammals. There is general agreement among researchers about the existence of age-related changes in certain neurochemical parameters, such as enzyme activity, receptor binding and neurotransmission. Biochemical evidence exists to support the existence of cholinergic dysfunction and cerebral phospholipid metabolism components in the physiopathology of cerebral aging (1, 4, 5). De Medio *et al.* (135) studied the effects of CDP-choline on changes in cerebral lipid metabolism during aging. They measured lipid synthesis *in vivo* in various areas of the brain in 12-month-old male rats. They injected a mixture of [2^3-H] glycerol and [Me-^{14}C]choline as lipid precursors into the lateral cerebral ventricle, and one hour after administration of the isotopes, they measured the incorporation of these precursors in total lipid, hydrosoluble intermediate and choline phospholipid fractions. In another series of tests, CDP-choline was injected intraventricularly into aged rats 10 min before sacrifice, performing the same radioactivity tests previously described. The distribution of radioactivity contained in the CDP-choline in the brain 10 min after its administration showed an enrichment in nucleotides and related hydrosoluble compounds in the areas studied. The incorporation of marked glycerol, which is very diminished in aged rats, was increased in all areas. The incorporation of marked choline also declines with age, but CDP-choline increased its uptake in the cortex. Consequently, the ^{3}H/^{14}C ratio increased in total lipids, in phosphatidylcholine and in choline plasmalogens after treatment with CDP-choline. Following this line of experimentation, López G.-Coviella *et al.* (136) studied the effects of oral CDP-choline on the cerebral phospholipid content in mice. These authors supplemented the animals' diet with 500 mg/kg/day CDP-choline for 27 months in 3-month-old mice and for 90, 42 and 3 days in 12-month-old mice, then measured phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine levels and the content of phosphatidylinositol plus phosphatidic acid in the cerebral cortex. After 27 months of treatment, the levels of phosphatidylcholine and phosphatidylethanolamine increased a significant 19 and 20%, respectively, while the level of phosphatidylserine increased 18%, although it did not reach statistical significance (Fig. 9). Similar elevations were observed when the 12-month-old mice were treated for 3 months, but not when they were treated for shorter periods of time. These results suggest that the chronic administration of CDP-choline can have important effects on the cerebral phospholipid composition, which could be responsible, in part, for the therapeutic efficacy of this drug. In addition, CDP-choline also benefits cerebral metabolism of nucleic acids and proteins (137-139), muscarinic and dopaminergic receptors (127) and neuroendocrine changes (140) in experimental models of aging. Due to these activities, the positive effects of CDP-choline on learning and memory in aged animals have been demonstrated in several studies (141, 142).

Experimental withdrawal and intoxication

When 300 mg of CDP-choline are injected intracarotidally in cats, effects analogous to those observed with the administration of 2 mg of morphine by the same route are obtained. The animal shows symptoms of rage and de-

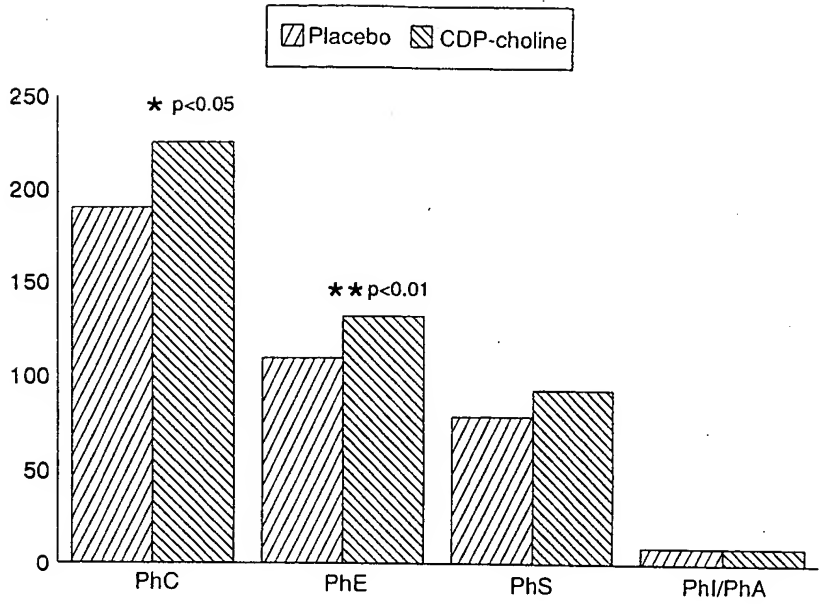

FIG. 9. Effects of chronic CDP-choline administration on cerebral phospholipid levels in 30-month-old mice that received a dietary supplement of CDP-choline (500 mg/kg/day) or placebo for 27 months. PhC: phosphatidylcholine; PhE: phosphatidylethanolamine; PhS: phosphatidylserine; PhI: phosphatidylinositol; PhA: phosphoric acid.

fensiveness, and its tail takes on a rigid, erect position. This phenomenon led to the idea that both substances may have some parallel effects on endogenous opium neuroreceptors and that the administration of CDP-choline could be useful in the treatment of opium withdrawal symptoms by slowing the effects of sudden withdrawal (143). Tornos *et al.* (144) studied the effects of CDP-choline on experimentally induced withdrawal using various tests, such as the jumping test in mice and study of behavior and changes in body temperature in rats. Naloxone-induced withdrawal in morphine-dependent mice was assessed by the number of jumps performed by the animals, showing a decline in intensity in the group of animals treated with 2 g/kg of CDP-choline, p.o., as compared to the control group of untreated animals. The diminished intensity of the withdrawal symptoms manifested itself in a 39% decrease in the average number of jumps performed by the animals in the 10 min following administration of the opium agonist. Analogically, in the behavioral study of morphine-dependent rats, it was proven that the administration of a 2-g/kg oral dose of CDP-choline administered simultaneously with naloxone was capable of significantly reducing the acute manifestations that characterize induced withdrawal. As for naloxone-induced hypothermia in morphine-dependent rats, the administration of a single oral dose of CDP-choline almost completely neutralizes this effect.

Delay in maturation and late development of dendrites in the neocortex, hippocampus and cerebellum are characteristic of alcohol-induced fetopathy. Based on these data, Patt *et al.* (145) carried out a study to investigate the effects of CDP-choline on Purkinje cells in newborn rats with alcoholic mothers. They took 70 Wistar rats born to 10 mothers subjected to alcohol intoxication for the entire gestation period and assigned them randomly to two treatment groups. One group of 35 animals received 100 mg of CDP-choline, i.p., for 3 weeks and the other group received the same quantity of saline solution for the same period. Seventeen animals were sacrificed at 13 days of age and 39 at 21 days. All the brains obtained were subjected to the Golgi impregnation technique

in order to investigate the effect of CDP-choline on Purkinje cells. The persistence of perisomatic processes and the degree of dendritic ramification served as qualitative criteria for Purkinje cell maturation and development; the total cell surface and the number of primary dendrites served as quantitative criteria. The degree of dendritic ramification and the number of mature cells, judging from the absence of persistent perisomatic processes, from the total surface area of Purkinje cells and from the number of primary dendrites (Fig. 10), were greater in the group of rats treated with CDP-choline. These differences were more significant in the 21-day-old than in the 13-day-old animals. Thus, the rats exposed to alcohol and treated with CDP-choline showed less delay in maturing and developing Purkinje cells, and this delay disappeared in the rats treated for 21 days with CDP-choline, which shows that this stabilizing agent of the neuronal membranes reduces the harmful effect of alcohol on the central nervous system. Petkov *et al.* (146) also demonstrated that CDP-choline reduces memory deficit in rats exposed pre- and postnatally to alcohol. CDP-choline has shown a similar protective effect against nicotine intoxication (147).

TOXICITY

Acute toxicity

Acute toxicity due to single-dose administration of CDP-choline has been studied in several animal species and with different routes of administration. The intravenous LD_{50} in mice, rats and rabbits is 4.6, 4.15 and 1.95 g/kg, respectively (148, 149). For oral administration, the LD_{50} is 27.14 g/kg in mice and 18.5 g/kg in rats (150). The LD_{50} of CDP-choline administered intravenously is 44 times higher than the LD_{50} of choline chlorhydrate at equivalent dosages, and it has been proven that at the same dosages of choline that produce cholinergic crises, CDP-choline does not produce any sign of toxicity (151, 152). This indicates that the administration of CDP-choline carries metabolic consequences which are clearly distinguished from those posed by the administration of exogenous choline.

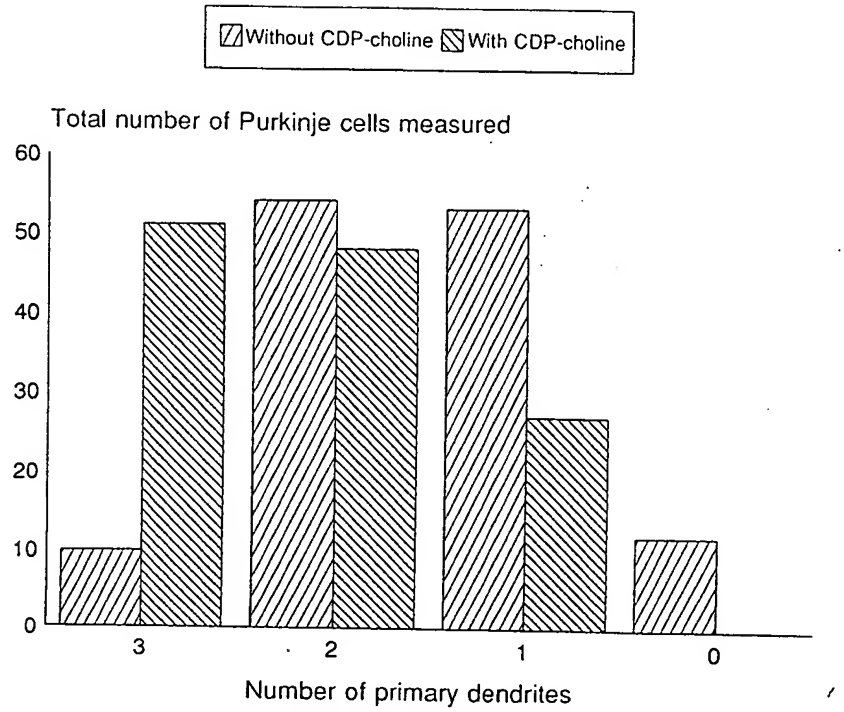

FIG. 10. Number of primary dendrites was used as the criterion for cellular development. Figure shows the differences observed in CDP-choline-treated or untreated 21-day-old rats exposed to alcohol.

Subacute toxicity

The intraperitoneal administration of a dose of up to 2 g/kg/day of CDP-choline for 4.5 weeks in rats did not yield any clinical signs of toxicity or significant changes in the hematological, biochemical or histological parameters analyzed. However, a slight reduction in food consumption and weight gain was observed after the second week of the study (150). Similar results were obtained from the subcutaneous administration of up to 1 g/kg/day for four weeks in male rats (149). The oral administration of 1.5 g/kg/day for 30 days did not cause histological, biochemical, hematological or ponderal changes in rats (153).

Chronic toxicity

The studies of chronic toxicity by the oral route (1.5 g/kg/day for 6 months in dogs) and the intraperitoneal route (1 g/kg/day for 12 weeks in rats) did not reveal significant anomalies related to the administration of the drug (149, 154). The intravenous administration of 300-500 mg/kg/day of CDP-choline for 3 months in dogs caused only toxic manifestations such as occasional vomiting, diarrhea and sialorrhea immediately after injection (152).

Teratogenicity

CDP-choline was administered to albino rabbits at a dose of 800 mg/kg during the phase of organogenesis, i.e., from the 7th to the 18th day of gestation. The animals were sacrificed on the 29th day and a detailed examination of the fetuses and their mothers was performed. No signs of maternal or embriofetal toxicity were observed. Effects on organogenesis were undetectable, and only 10% of the treated fetuses showed a slight lateness in cranial osteogenesis (unpublished data).

PHARMACOKINETICS

Plasma level curves: Bioavailability

Marked CDP-choline [methyl ^{14}C] was administered at a dose of 4 mg/kg by injection into the jugular vein, and orally, by nasal-gastric probe (155). The results obtained, expressed as a percentage of the radioactivity in 10 ml of blood for each of the administration routes, are shown in Table 4. The bioavailability of the oral route of administration as compared to the intravenous route was calculated from these data, with the finding that it was practically complete, which is consistent with the fact that, in the same study, no residual radioactivity was found in the feces eliminated in the 72 hours after oral administration.

López G.-Coviella *et al.* (156) studied the effects of CDP-choline on the plasma levels of cytidine, choline and CDP-choline in healthy volunteers who received the substance orally or intravenously, and in rats who received it intravenously. Two hours after administration of a single 2-g oral dose of CDP-choline, the plasma levels of choline increased 48% and those of cytidine, 136% (Fig. 11). In the individuals who received three 2-g doses at 2-hour intervals, plasmatic choline reached a peak, approximately 30% from the baseline, 4 hours after the administration of the initial dose of CDP-choline, whereas plasma levels of cytidine increased for up to 6 hours (Fig. 12) and were 5 times the base value (p <0.001). The CDP-choline administered intravenously was rapidly

TABLE 4. Kinetics of total radioactivity in the blood after the oral or intravenous administration of 4 mg/kg [methyl ^{14}C]CDP-choline in male rats. Percentage of total radioactivity (mean±SD) is shown with respect to total administered.

Time	Oral	Intravenous
10'	0.26±0.12	3.05±0.24
20'	0.40±0.02	2.59±0.31
30'	0.74±0.01	1.47±0.22
1h	1.32±0.40	1.40±0.02
2h	2.33±0.63	2.84±0.02
3h	3.31±0.86	2.50±0.05
4h	3.57±0.88	2.77±1.00
5h	4.17±0.83	3.37±0.31
6h	4.18±0.03	3.68±0.02
7h	3.81±0.73	–
24h	2.48±0.40	3.12±0.19

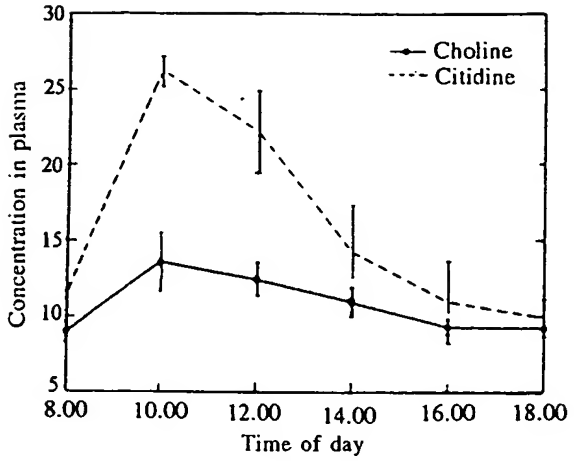

FIG. 11. Plasma concentrations of choline and citidine immediately after the administration of a single 2-g oral dose of CDP-choline in humans.

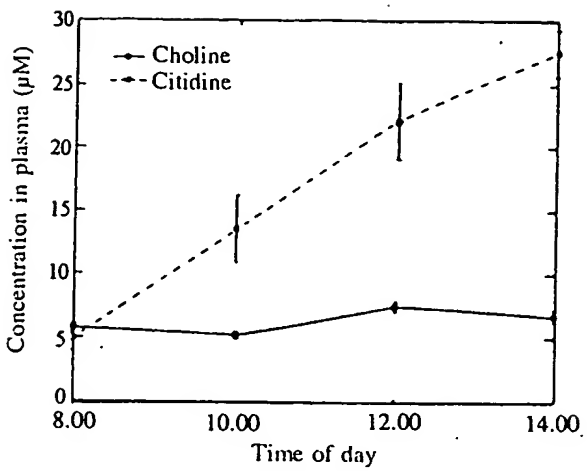

FIG. 12. Plasma levels of choline and citidine after the administration of 3 consecutive 2-g oral doses of CDP-choline in humans.

hydrolyzed, in both humans and rats. In healthy individuals who received an infusion of 3 g CDP-choline in 500 ml of physiological saline solution for 30 min, the CDP-choline levels were almost undetectable immediately after finalizing the infusion period, a moment in which the plasma levels of cytidine and choline reach a peak, although their concentration remained significantly elevated for up to 6 hours after the start of infusion (Fig. 13). These observations show that CDP-choline, administered both orally and intravenously, is converted into two principal circulating metabolites: cytidine and choline.

Diffusion in tissue and distribution: Transport and metabolism

The diffusion in tissue of CDP-choline and its metabolites has been studied in rats administered intravenous [methyl $5^{14}C$,5-^{3}H]CDP-choline, marked in the choline and cytidine fractions (157, 158). In the same battery of tests, plasma radioactivity levels were determined for the marked metabolites during the

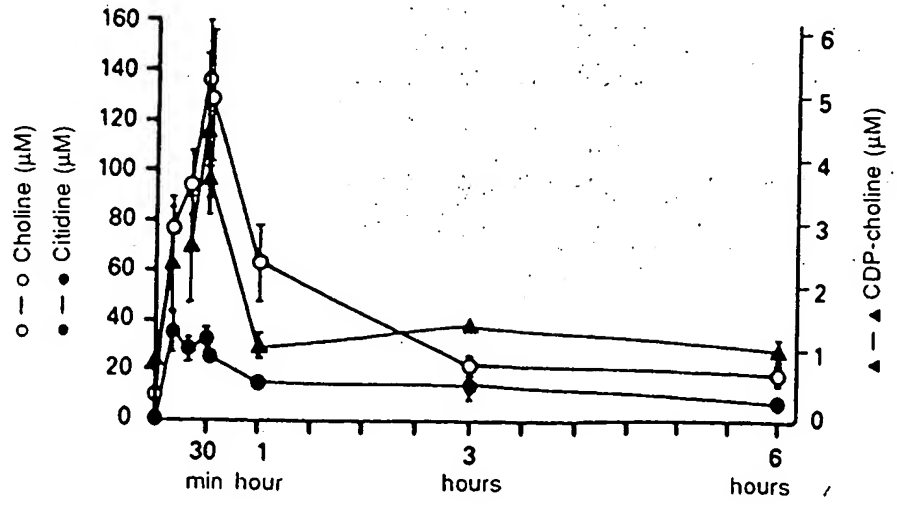

FIG. 13. Concentrations of choline, citidine and CDP-choline in human plasma after the intravenous infusion of a CDP-choline solution (3 g/500 ml of physiological saline solution).

first 30 min following administration, and renal and fecal excretion levels, during the first 48 hours. Only 2 min after the injection, less than 10% of the administered radioactivity was found in plasma. Furthermore, the radioactivity excreted by the kidneys during the first 48 hours represented only 2.5% of the ^{14}C and 6.5% of the ^{3}H administered. In the same interval, fecal excretion did not exceed 2% of the dose administered. These results suggest that after its administration, CDP-choline is distributed rapidly into tissues and used by them. Figure 14 shows radioactivity levels found in the liver, brain and kidneys, at different times after the intravenous administration of double-marked CDP-choline. The evolution of levels in the brain is of particular interest; the incorporation of radioactivity in this organ increased progressively for the first 10 hours after administration of the drug and the levels reached still persisted, without decreasing, at 48 hours.

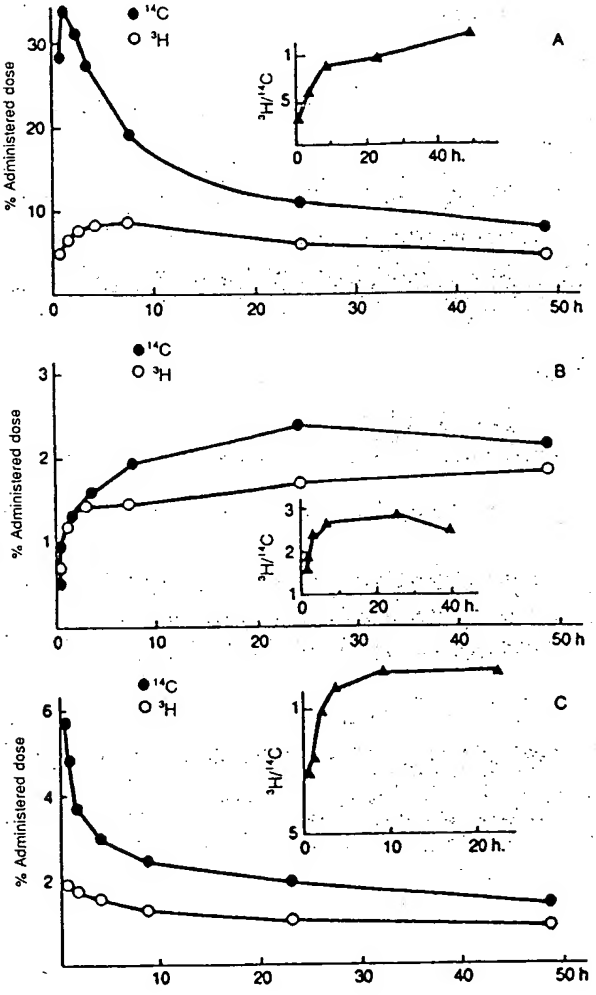

FIG. 14. Radioactivity levels in the liver (A), brain (B) and kidneys (C) of rats at different times following the injection of double-marked CDP-choline at a dose of 2 mg/kg. All values represent the average obtained from 10 animals.

In one group of animals, levels of radioactivity corresponding to the marked compounds present in the brain were determined at 0.5, 1, 4 and 48 hours after the administration of double-marked CDP-choline. It was observed that the cerebral radioactivity corresponding to the ^{3}H was at first concentrated primarily in cytidinic nucleotides, then later in nucleic acids. With respect to the ^{14}C-marked compound, the highest levels at first corresponded to betaine, choline and phophorylcholine, but at 4 hours, [^{14}C]methionine and [^{14}C]phospholipids represented 26.4 and 24.2%, respectively, of the total cerebral radioactivity corresponding to ^{14}C. After 48 hours, this radioactivity was concentrated principally in phospholipids and proteins. Thus, it was observed that the marked phospholipids increased continuously during the 48 hours after administration of double-marked CDP-choline. As shown in Figure 15, this increase was rapid in the first 5 hours, then slower thereafter.

In another battery of tests, after the administration of [methyl ^{14}C]CDP-choline, the presence of this drug was determined in various areas of the brain and its distribution in cere-

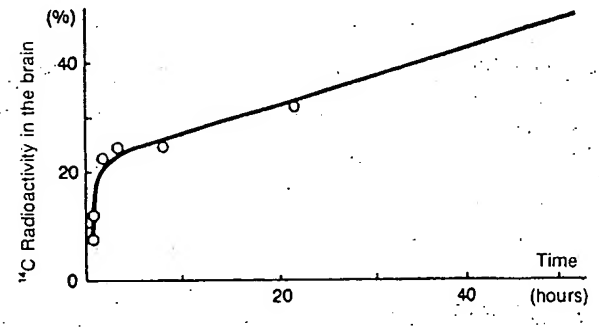

FIG. 15. Evolution of ^{14}C-phospholipid levels in rat brain following the intravenous administration of double-marked CDP-choline. The levels represent averages from 3 rats and are expressed as a percentage of the total radioactivity corresponding to ^{14}C in the brain.

bral ultrastructures measured (159-163). In a study carried out with high resolution autoradiography in mouse brain, it was observed that 24 hours after the administration of marked CDP-choline, the radioactive marker was incorporated widely in the various brain areas studied (cerebral cortex, white matter and central grey nuclei); it was found in both intra- and extracellular spaces, with a notable presence in cell membranes. Using the same experimental model, but 10 days after administration of the marked drug (160), a concentration of radioactivity was observed in the most myelinated areas, as well as a notable incorporation by cerebral Purkinje cells. Using low resolution autoradiography, the distribution of marked CDP-choline radioactivity was analyzed in rat brain 5 and 24 hours after administration of the drug (161), finding that at 24 hours most of the radioactivity was detected at the intracellular level. In another study, the incorporation of radioactivity from [methyl ^{14}C]CDP-choline was analyzed in the various cerebral phospholipid fractions after its oral administration in male Sprague-Dawley rats (162). Of the total radioactivity, 62.8% was found forming part of the cerebral phospholipids, especially phosphatidylcholine and sphingomyelin, which shows that oral CDP-choline has an effect on the synthesis of structural phospholipids of the cell membranes. These results are in agreement with those obtained by Aguilar *et al.* (163), who demonstrated that the radioactivity from marked CDP-choline was associated with mitochondrial and cytoplasmic membranes in brain homogenate.

In conclusion, these studies demonstrate that CDP-choline is widely distributed in cerebral structures, with a rapid incorporation of the choline fraction in structural phospholipids and of the cytosine fraction in cytidine nucleotides and nucleic acids. CDP-choline reaches the brain and is actively taken up in mitochondrial, cytoplasmic, and cell membranes, forming part of the structural phospholipid fraction.

Elimination pathways and kinetics

When marked CDP-choline is administered, either orally or intravenously, the elimination of radioactivity is very slow; the elimination follows urinary, fecal or expired CO_2 routes (164).

Total radiation excretion 5 days after oral administration of [^{14}C]CDP-choline to healthy volunteers is shown in Figure 16. Table 5 presents the principal data relevant to the kinetic elimination of the drug.

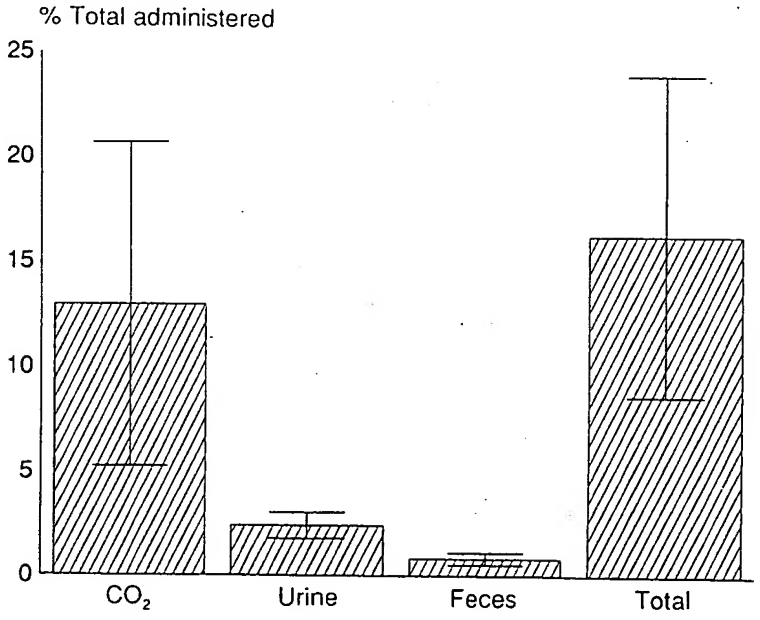

FIG. 16. Total excretion of radioactivity (percentage of the total administered) for 5 days following the oral administration of [^{14}C]CDP-choline. Values expressed as averages for 6 individuals.

TABLE 5. Most significant parameters of the kinetics of the elimination of [^{14}C]CDP-choline after oral administration. Data express mean for 6 individuals.

Parameter	CO_2	Urine	Feces
Maximum excretion velocity (% dose/h)	1.22±0.59	0.159±0.084	0.021±0.008
Time to maximum excretion (h)	1.60±0.73	1.3±0.8	56±18
First phase of elimination			
Apparent half-life (h)	2.58±0.60	6.62±1.28	–
Apparent elimination velocity (% dose/h)	0.279±0.055	0.107±0.017	–
Second phase of elimination			
Apparent half-life (h)	56.22±33.39	71.08±58.16	19.39±6.63
Apparent elimination velocity (% dose/h)	0.030±0.049	0.013±0.006	0.039±0.014

In the urinary elimination of the drug, two phases can be discerned: the first phase, which lasts about 36 hours, shows a rapid decline in the speed of excretion; in the second phase, the speed of excretion declines much more slowly. The same goes for expired CO_2, whose elimination speed decreases very rapidly in the first 15 hours, approximately, declining more slowly thereafter.

CLINICAL EXPERIENCE

Head injury and sequelae

In the experimental studies previously described, it was shown that the administration of CDP-choline gives rise to a significant regression in cerebral edema and an improvement in EEG and in the alteration in consciousness, as well as in the quality of survival. The effect on the level of consciousness is attributable to the facilitating action of the electroencephalograph reaction upon waking, caused by the stimulation of the ascendent activative reticular system in the brain stem.

Using these experimental premises, many clinical studies have been carried out to determine whether these effects have any implications in the treatment of patients with head trauma.

In 1967, Moriyama *et al.* (165) published a study on the effects of CDP-choline on 25 patients with head injury and depressed level of consciousness, demonstrating its efficacy in improving neurological clinical symptoms and state of consciousness in 70% of the cases and, in addition, showing the perfect tolerance of the product, with not a single side effect being observed.

Ayuso and Saiz (166) carried out a double-blind study on the usefulness of CDP-choline in bilateral electroshock-induced memory dysfunction in a series of 22 patients admitted for endogenous depression. The group that received the active medication showed a smaller reduction in memory performance after four electroshock sessions, as compared to the control group, proving the usefulness of citicoline in the treatment of patients with organically based memory disturbances.

De la Herrán *et al.* (167) compared the effects of the administration of citicoline in a group of 50 patients with impairment in consciousness level (which was of traumatic origin in 32 cases) with another group of patients with similar characteristics who received the usual treatment. Table 6 shows the characteristics of coma in these trauma patients and the percentage of positive results obtained as a function of the depth of coma; 34% of the patients recovered consciousness in the first 48 hours. A few days later, 66% of the patients

TABLE 6. Coma level as related to final result.

Coma level	I	II	III	IV
Patients (n)	13	12	6	1
Good result (%)	93.7	80	12.5	0

had recovered consciousness, showing a higher percentage than in the control group. Results such as these demonstrate that CDP-choline reactivates and accelerates the normalization of state of consciousness in patients with head injury.

Carcassonne and LeTourneau (168) carried out a double-blind study on a group of 43 children with true trauma-induced disturbances in consciousness, after ruling out serious cases and those which required surgical treatment. After analyzing the results obtained, these authors reached the conclusions that CDP-choline:

- Is perfectly tolerated, both locally and generally.
- Accelerates the recovery of a normal state of consciousness.
- Speeds the disappearance of neuropsychological disorders and of disturbances in cerebral electrogenesis.
- Confers a better quality in the patients' evolution.

Espagno *et al.* (169) compared the effects of citicoline versus placebo in a group of 46 patients who had suffered a head injury. In a double-blind study, 22 patients received 250 mg/day of citicoline parenterally for 20 days and 24 patients received placebo. The results obtained showed that in slight coma, CDP-choline significantly accelerated (p <0.05) the recovery of consciousness, whereas in more severe coma and at the dosage administered – a dose which is now considered very inadequate – citicoline improved the prognostic, with 75.2% of the patients in the group administered placebo presenting a late recuperation (>15 days) of consciousness and/or evolution of *excitus*; in contrast, in the group treated with the active drug, recovery from coma later than the 15th day occurred in 31% of the cases and the incidence of prolonged coma and/or *excitus* was 12.5% In conclusion, CDP-choline conditioned an earlier recovery of consciousness and a greater number of EEG and clinical improvements, and was, notably, perfecty tolerated.

Richer and Cohadon (170) carried out a double-blind study on 60 coma patients with traumatic etiology. The patients were divided into two groups; one group received the active drug and the other, placebo. In terms of the duration of coma, after 60 days the number of patients who had recovered consciousness was significantly higher (p <0.01) in the group treated with CDP-choline. After 90 days, there was a greater recuperation (p <0.04) from motor impairment in the group treated with citicoline. It was also shown that the recuperation of walking was significantly accelerated in the treated group. Consequently, after 60 days, a greater socioprofessional reintegration (p <0.06) was seen in the group treated with CDP-choline. Thus, the limiting effect of citicoline on the duration of posttraumatic coma was made evident, as was the drug's participation in recovery from deficits associated with the encephalic lesions that accompany these comas. In contrast, mortality was not changed by the treatment.

In a double-blind test, Lecuire and Duplay (171) compared the effects of citicoline, at a dose of 750 mg/day, i.v., with those of meclophenoxate, at a dose of 3 g/day, i.v., in a group of 25 patients. Analysis of the results showed a significant improvement in the group of patients treated with citicoline, above all with respect to the recovery of consciousness, EEG changes and functional recovery. The mean duration of coma was 10 days in the group treated with citicoline and 20 days in the group treated with meclophenoxate. After 10 days, the EEG had improved in 50% of the patients treated with citicoline and in 18% of those who received meclophenoxate. In conclusion, citicoline was found to be superior to meclophenoxate. CDP-choline's principal characteristic was accelerated recovery of consciousness level, which is related to the improved EEG results. These same authors carried out an open study on a series of 154 patients with head injury (172) in which they assessed the effects of treatment with CDP-choline, finding that it speeded awakening and the recovery

from deficit symptoms, as well as improving the quality of survival.

Subsequently, Lecuire (173) carried out a double-blind, comparative study between piracetam (6 g/day) and citicoline (750 mg/day) in a group of 40 patients with head injury, finding 75% favorable evolutions in the group of patients who received citicoline compared to 33% in the group treated with piracetam.

Cohadon *et al.* (14, 75, 174) proved the clinical efficacy of citicoline in a double-blind study carried out on 60 patients with severe cranial trauma. In both groups, standard treatment was followed and surgery was performed when necessary. One group of patients received citicoline at a dose of 750 mg/day, i.v., for the first 6 days and later intramuscularly for 20 additional days; the other group received placebo. The clinical evaluation was continued for 6 months. After 15 days, the response to pain stimulus was greater in the group of patients treated with CDP-choline ($p < 0.01$), and an earlier recovery of consciousness was obtained in this group (Fig. 17). A greater recovery from neurological impairment was also found in the treated group. After 120 days, 84% of the patients in the group treated with CDP-choline were able to walk unassisted, while 62.5% in the placebo-treated group had recovered this ability, with a statistically significant difference ($p < 0.01$) from day 60 onwards. Table 7 shows the final results obtained for both groups as assessed by the Glasgow Orientation Scale (GOS); mortality rates were similar. The data obtained in this study show that CDP-choline shortens the time required for the recovery of consciousness and speeds the recovery from neurological deficits in patients with severe head trauma.

TABLE 7. Final results for each treatment.

	Glasgow Recovery Scale				
Group	I	II	III	IV	V
Placebo	12	5	4	3	6
CDP-choline	11	9	3	2	5

Deleuze *et al.* (175) demonstrated that citicoline is capable of reducing serum levels of creatine phosphokinase (CPK) and lactate levels in cerebrospinal fluid, with a decrease in the lactate/pyruvate ratio, in patients with severe cerebral suffering and coma. The product's perfect tolerability was also noteworthy.

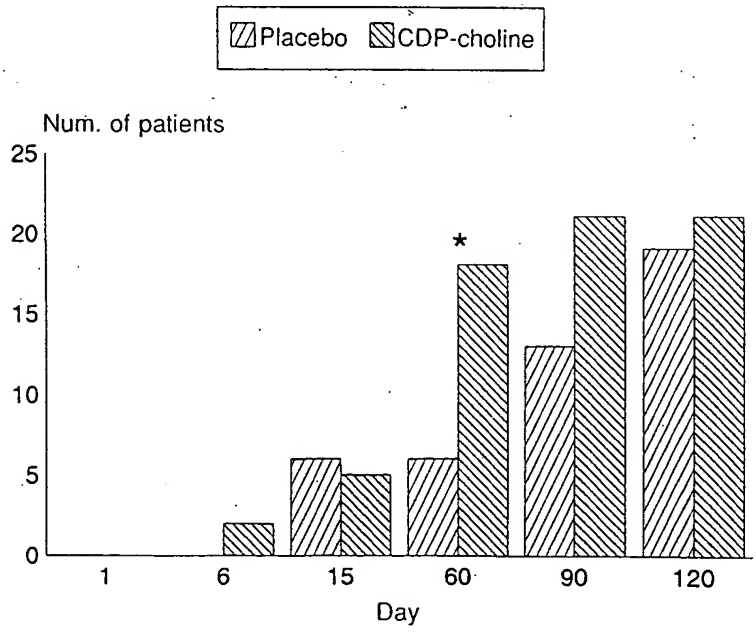

FIG. 17. Normalization of consciousness level with respect to time and treatment received. *$p < 0.01$.

Ogashiwa *et al.* (63) carried out a clinical test in 101 patients with disturbances in consciousness level with varying etiology (20% with traumatic causes), showing the efficacy of citicoline in improving the General Recovery Rate, which is closely related to the Principal Component Analysis Score. They found that citicoline was more effective in items related to the executionary factor than in those related to the verbal factor, and that the best effects obtained were with patients under 60 years of age with a stabilized period of impairment not exceeding 3 weeks. The excellent tolerability of the product is noteworthy; it is even administered intrathecally in some cases (176, 177).

At the Neurosurgery Services of the "Ramon y Cajal" Special Center in Madrid, a series of 100 patients with head trauma treated with CDP-choline until their release from the center was compared with another series of 100 individuals with similar characteristics who did not receive citicoline (178). The treatment with citicoline was started at a parenteral dose of 600-1200 mg/day, switching to 300-900 mg/day, p.o., during the rehabilitation stage. Evolution was followed by assessing the mean duration of coma, the persistence of psychological and neurological symptoms, the WAIS test and electrophysiological tests of muscular tension. The results obtained indicated that the addition of CDP-choline to the treatment protocol caused a decrease in the duration of post-traumatic coma and sequelae, both neurological and psychological, attaining the best response in the recovery from disturbances in the intellectual sphere (Fig. 18) and in motor deficit (Fig. 19).

In a national survey taken in France, Ragueneau and Jarrige (179) collected 921 cases of severe head injury, i.e., with an initial score on the Glasgow Coma Scale (GCS) of less than or equal to 8. Of these patients, 219 had received treatment with citicoline, which allowed the establishment of two groups and the comparison of their results. No significant differences were found with respect to mortality, but with respect to the number of dependent states, the differences were significant, with the greatest effect being in patients with an initial GCS of 6-7 (Table 8). Citicoline improved the quality of survival, permitting a more frequent reintegration into social and family life, as well as a return to work or school. Head injury-induced mortality essentially depends on the initial lesions, which, except in the case of epidural hematoma, are outside of all real therapeutic solutions.

Calatayud *et al.* (180) presented the results of the influence of CDP-choline incorporation in the treatment of head injury. They gathered

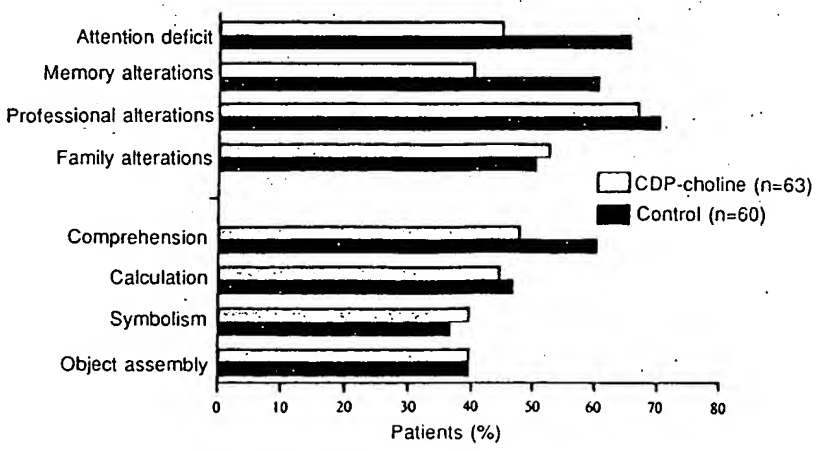

FIG. 18. Percentage of patients with psychological sequelae after 6 months of treatment (patients with subreactivity II or III).

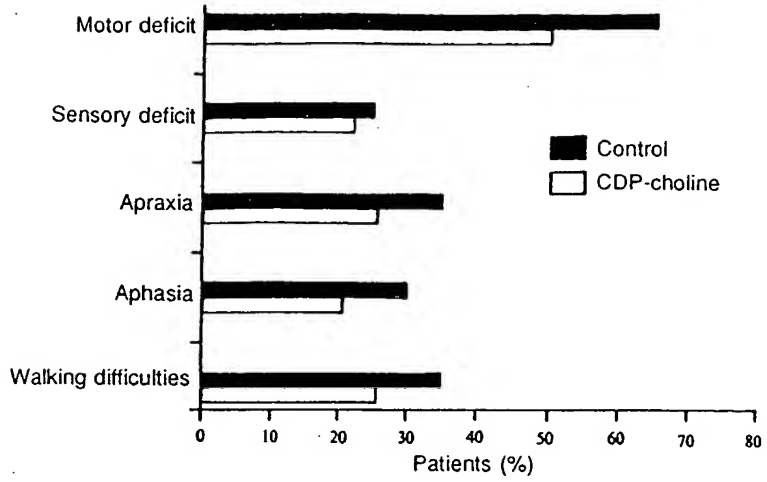

FIG. 19. Percentage of patients with neurological manifestations after 6 months of treatment (patients with subreactivity II or III).

TABLE 8. Impact of CDP-choline on the final results. Results expressed as percentages.

Result	With CDP-choline (219 patients)	Without CDP-choline (702 patients)
Exitus	44	43
Vegetative state	5	6
Dependent state	7*	15
Independent state	44*	37

*p <0.001 compared to the group of patients not treated with CDP-choline.

together 216 patients with an initial GCS of between 5 and 10, of whom 115 received treatment with citicoline. The average dose of citicoline administered was 4 g/day. After analyzing the results, it was found that citicoline:

- Reduces hospital stay (p <0.05) and the duration of outpatient care (p <0.001), with these differences being more marked in the group of patients with an initial GCS of between 5 and 7 (Fig. 20).
- Favors the recovery of memory, motor impairment, superior neurological functions and character changes, and improves the global functional result (Table 9).

Lozano (181) examined the repercussions of treatment with citicoline in the evolution of posttraumatic cerebral edema in a study carried out on 78 head injury cases who had an initial GCS of between 5 and 7. In all cases, cranial computed tomography (CT) was performed at the beginning and end of the study in order to assess the evolution of the CT of cerebral edema. Other parameters analyzed included duration of hospital stay and degree of autonomy at the moment of release from the hospital.

Citicoline was administered to 39 patients at a dose that oscillated between 3 and 6 g/day by continuous intravenous perfusion for the first two weeks. After 14 days of treatment with citicoline, the CT of cerebral edema had evolved as shown in Figure 21. It was observed that the cerebral edema was reduced or normalized in a greater number of patients in the group treated with citicoline than in the control group, with these differences being highly significant (p <0.005). No significant differences were observed between the two groups in either the therapeutic requirements or treatment received. The average hospital stay was 28.718±21.6 days for the group that had received the active treatment and 37.323±35.22 days for the control group, with these differences being statistically significant (p <0.001). In the final results, which were assessed using the Glasgow Orientation Scale (GOS), the differences did not attain statistical significance due to the small number of cases and the spe-

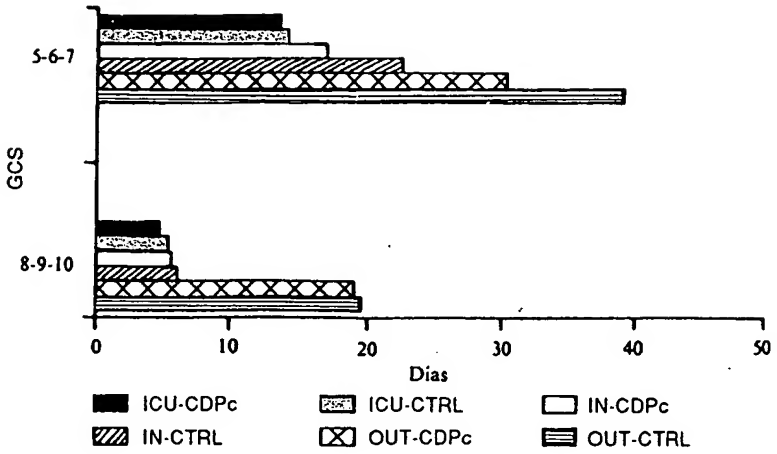

FIG. 20. Mean hospital stay (in intensive care unit and in-patient care) and duration of out-patient care with respect to initial Glasgow Coma Scale (GCS) and treatment with CDP-choline. ICU: intensive care unit; IN: in-patient care; OUT: out-patient care; CTRL: control; CDPc: CDP-choline.

TABLE 9. Final results, assessed with the Glasgow Recovery Scale (GOS), as a function of treatment received ($p < 0.05$).

GOS	CDP-choline	Control
I	77	51
II	19	31
III	1	7
IV	0	2
V	18	10

cial characteristics of this type of patients. However, a tendency towards a more favorable resolution was observed in the group of patients treated with CDP-choline (Table 10).

Levin (182) carried out a study on 14 patients with postconcussion syndrome after mild or moderate cranial trauma. This syndrome is characterized principally by the presence of symptoms such as headache, vertigo, memory disturbances and alterations in sleep. In this

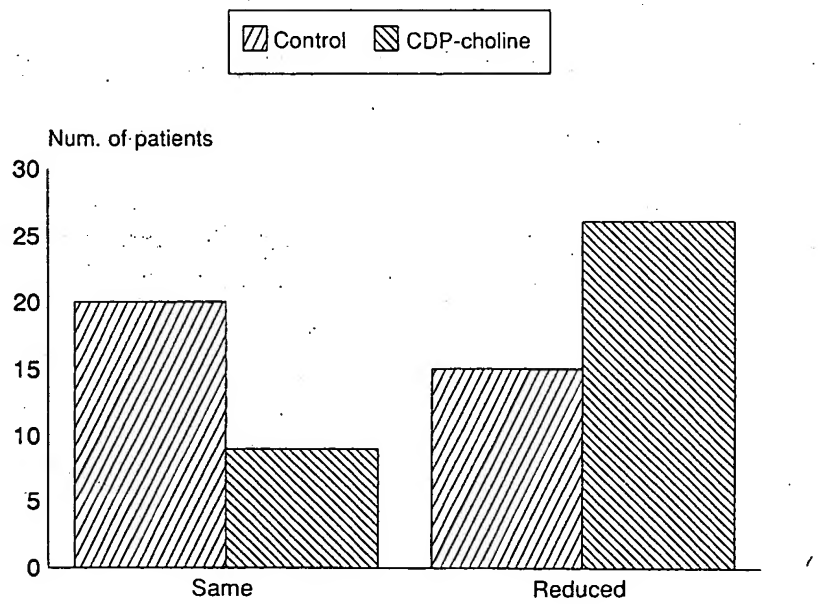

FIG. 21. Evolution of the CT of cerebral edema after 14 days of treatment.

TABLE 10. Final results, assessed with the Glasgow Recovery Scale (GOS), as a function of the treatment received (n.s.).

GOS	CDP-choline	Control
I	15	11
II	8	8
III	6	7
IV	5	6
V	6	7

study, patients treated with CDP-choline for one month showed an improvement in memory tests (especially in those dealing with recognition) that was statistically significant as compared to the group treated with placebo. In Figure 22, the evolution of symptoms after one month of treatment is shown; the improvements obtained in the patients treated with CDP-choline were greater than in those treated with placebo, except for gastrointestinal discomfort. The sensation of vertigo was significantly more frequent in the patients treated with placebo after one month of study.

As a final conclusion, we can say that it was more than sufficiently demonstrated that patients with head trauma, especially those with an initial GCS of 5-7, benefit from the incorporation of CDP-choline into the therapeutic scheme, because it accelerates the reabsorption of cerebral edema and speeds recovery, both in terms of consciousness and in terms of neurological disturbances, which translates into shorter hospital stays and a higher quality of survival. Furthermore, in cases of mild or moderate head injury, CDP-choline significantly reduces the duration and intensity of post-concussion syndrome.

Acute cerebral vascular disease and sequelae

As we have seen experimentally, CDP-choline is a drug which has pleiotropic activities which imply the activation of the neuronal metabolism, the stabilization of neuronal membranes and their function, and the normalization of neurotransmission.

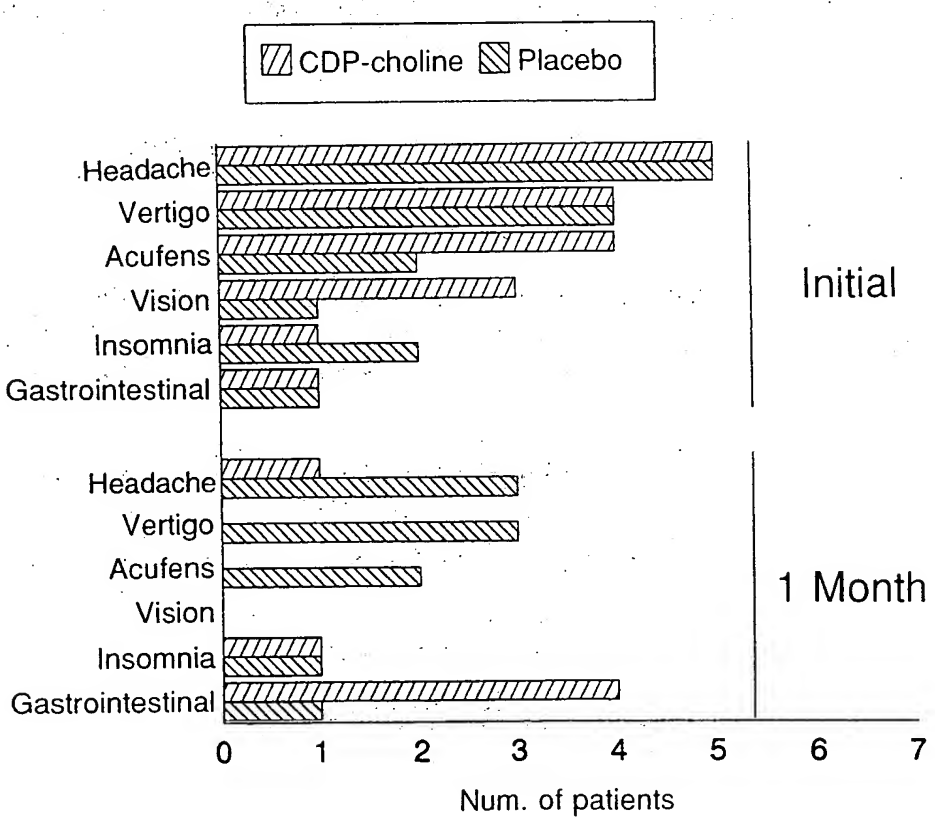

FIG. 22. Evolution of postconcussion symptoms after one month of treatment with CDP-choline or placebo. The number of patients that reported each of the alterations above is shown.

In the 1960s, various studies were carried out in which CDP-choline's efficacy in reducing the neurological symptomatology of cerebral ischemia was discerned (183, 184).

Hazama *et al.* (185) carried out a double-blind study to evaluate the effect of CDP-choline on the functional recovery of hemiplegia in 165 patients with cerebral vascular disease. These authors showed that CDP-choline at a dose of 1000 mg/day for 8 weeks was superior to placebo, especially in motor recovery in the upper extremities, concluding that this drug promotes a natural recuperation from hemiplegia.

Goas *et al.* (186) carried out a double-blind, comparative study of CDP-choline versus placebo on 64 patients with cerebral infarct of less than 48 hours of evolution. In the assessment carried out at the end of 3 months, it was demonstrated that CDP-choline was superior to placebo in the improvement of motor deficit ($p < 0.05$), hypertonia ($p < 0.03$), recovery of walking ($p < 0.02$), and the evolution of EEGs ($p < 0.01$) and psychometric tests ($p < 0.05$), yielding a higher number of independent states (51.6% with CDP-choline *vs.* 24.24% with placebo) (Fig. 23).

Tazaki *et al.* (187) carried out a double-blind, multicenter, controlled preliminary study with placebo on the usefulness of CDP-choline in the treatment of acute-phase cerebral infarction. Sixty-three Japanese academic centers, including a total of 272 patients selected with strict inclusion criteria, participated in this study. The patients were randomly distributed and received either 1 g/day of CDP-choline or saline solution (placebo), i.v., for 14 days. At the end of treatment, it was proven that CDP-choline significantly improved consciousness (51% vs. 33% for placebo; $p < 0.05$), global improvement rates (52% vs. 26%; $p < 0.01$) and global utility rates(47% vs 24%; $p < 0.001$). In addition, the group of patients treated with CDP-choline had fewer complications (1%) than the group treated with placebo (8.1%). These authors concluded that CDP-choline is a safe, effective drug for the treatment of acute-phase cerebral infarction. These results

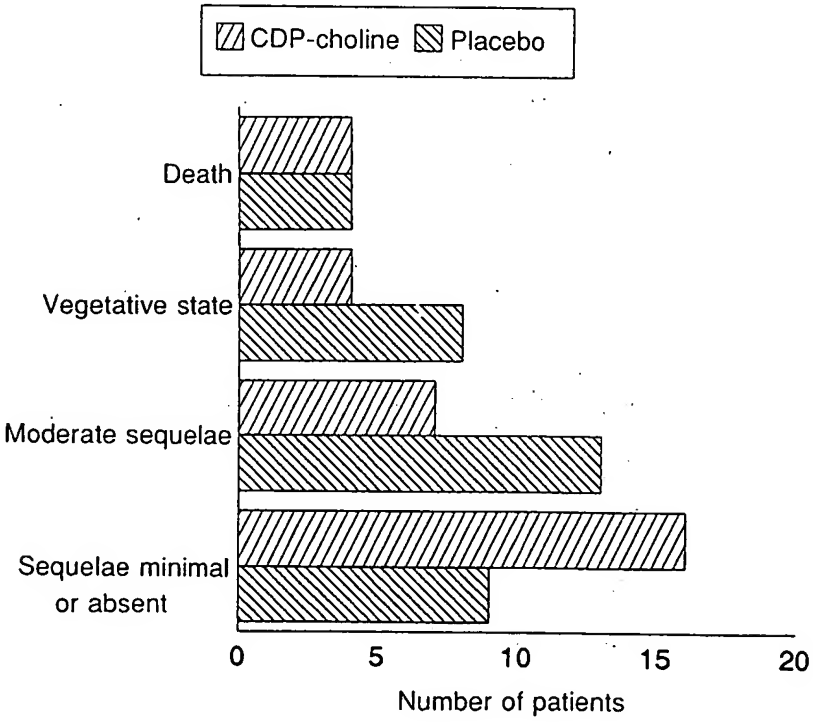

FIG. 23. Final results obtained with respect to treatment received. A larger number of good results are shown for CDP-choline in comparison with the control group.

are consistent with those obtained by other authors (188-191).

Recently, Guillén (192) carried out a random comparative study on the efficacy of CDP-choline vs. conventional therapy in the treatment of acute-phase ischemic stroke in order to determine whether CDP-choline's activity would attenuate the histological and neurological changes found in cerebral ischemia. The study gathered information from a sample of 80 subjects of both sexes, over 65 years of age, who were in the acute phase of ischemic stroke as confirmed by CT, who had initiated treatment less than 48 hours after the start of symptoms, and who had a slight or moderate alteration of consciousness, with a score greater than or equal to 10 on the Glasgow Coma Scale and/or a score of between 30 and 80 on Mathew's Neurological State Scale upon initiation of treatment. The 80 patients were randomly distributed in two groups of 40 patients; one of the groups received treatment with CDP-choline, while the other received only conventional treatment. All patients received standard treatment for 5 days (500 ml of saline solution and 10% dextran glucose, i.v., daily). One group was treated with CDP-choline (1 g/8 h in daily i.v. infusion for 10 days) and the other constituted the control group. However, both groups were treated with fluid maintenance therapy and low-dose heparin, following the researcher's criteria. The duration of CDP-choline treatment was 10 days. In the group treated with CDP-choline, the score on the Glasgow Coma Scale was 12.55±2.13 initially and 13.85±1.09 at the end of treatment (p <0.005). In the control group, this score went from 12.2±1.75 to 12.36±1.64, but these differences were not statistically significant (Fig. 24). An analysis of the differences between groups found that, in the final assessment, the scores obtained in the group treated with CDP-choline were statistically higher for this scale than those of the control group (p <0.05). With respect to the Mathew's Scale, in the group treated with CDP-choline, the score went from 64.13±18.55 to 76.72±16.16 at the end of the active treatment period (p <0.001). In the control group, a statistical improvement was also observed in these scores, moving from an initial score of 61.83±14.72 to a final score of 65.16±16.53 (p <0.001). An analysis comparing the groups demonstrated that the scores obtained with this

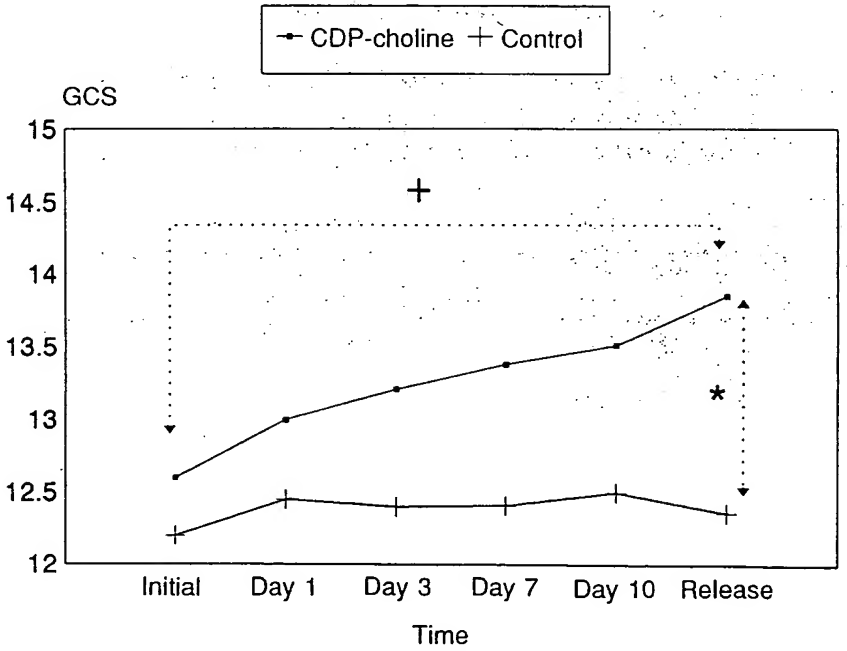

FIG. 24. Evolution of Glasgow Coma Scale (GCS) scores with respect to treatment received. *p <0.05; +p <0.005.

scale after 10 days of treatment were significantly higher in the group treated with the drug being studied (p <0.01). The score obtained in the Barthel scale also improved significantly in both groups. In the group treated with CDP-choline, the scores moved from a baseline value of 22.25±20.03 to a final value of 49.0±32.52 (p <0.001). In the control group, the scores changed from 19.88±21.59 to 38.5±37.32 (p <0.001). An analysis comparing the groups showed a better improvement in the scores obtained with this scale in the group treated with CDP-choline, with the differences being statistically significant (p <0.05). The Red Cross Physical Disability Scale improved in both groups. In the group treated with CDP-choline, the base score was 3.7±0.88 and the final score was 2.53±1.41 (p <0.05). In the control group, these scores were 3.68±0.99 and 2.90±1.54, respectively (p <0.05). An analysis comparing the two groups showed that the improvement obtained in the group treated with CDP-choline was significantly higher than in the control group (p <0.05). The Red Cross Psychological Disability Scale also improved in both groups. In the group treated with CDP-choline, the baseline score was 1.45±1.17 and the final score was 0.81±1.05 (p <0.001). In the control group, the score went from 1.8±1.26 to 1.85±1.31 (p <0.001). An intergroup analysis showed that the improvement obtained in the group given CDP-choline was significantly greater than that of the control group (p <0.05).

In conclusion, it can be asserted that patients with cerebral infarct in the acute phase, as well as in the sequela phase, benefit from treatment with CDP-choline and attain a better functional and neurological recovery, and that, furthermore, this treatment is safe and well tolerated.

Cognitive Disturbances

In the last several years, various experimental investigations of cerebral aging have led to giving an increasing importance to changes in neuronal metabolism as a participating factor in the physiopathology of this process. In the aging brain, there is a general decline in enzymatic activities associated with energy metabolism, as well as more specific biochemical changes that affect nucleic acid and lipid metabolism. It has also been demonstrated that, in both aging processes and in certain senile and presenile diseases, there is a link with certain neurotransmitters (e.g., dopamine, acetylcholine) and hormones (e.g., growth hormone, prolactin).

As demonstrated in the various experimental studies analyzed, CDP-choline increases phospholipid synthesis and glucose uptake in the brain in conditions in which they have been diminished. It also influences neurotransmitter metabolism, since it has been demonstrated that it increases dopamine synthesis in certain regions of the brain. Based on these facts, many clinical tests have been carried out to assess the efficacy of CDP-choline in the treatment of cognitive disorders associated with cerebral aging, chronic cerebral vascular disease and dementia.

In one of the first studies carried out in this field, Madariaga *et al.* (193) showed that treatment with CDP-choline of a group of senile women induced an improvement in memory, cooperation and ability to interact with surroundings. Fassio *et al.* (194) discuss the usefulness of CDP-choline in psychogeriatrics, highlighting that its application as an in-depth treatment allows a reduction in the dosage of the drugs normally used in psychogeriatrics. The usefulness of CDP-choline in the treatment of senile cerebral involution has been demonstrated to decrease the characteristic symptomatology in many studies (195-204). In a controlled, open study carried out on a group of 30 patients with senile involutional cerebropathy, Lingetti *et al.* (195) obtained an 83.3% improvement in symptoms, with a notable lack of side effects associated with the treatment. Stramba-Badiale and Scillieri (196) were able to demonstrate a significant improvement in the scores of Fishback's Mental Status Questionnaire in a group of 24 elderly subjects after 20 days of treatment with 500 mg/day, i.m., of CDP-choline. Bonavita *et al.* (197) highlight the efficacy

TABLE 11. Percentages of remission and improvement in symptoms (p <0.001 for each of the symptoms with respect to the initiation of treatment).

Symptom	N° of patients	% Remission	% Improvement
Mood	1521	38.2	40.9
Emotivity	1559	36.9	39.7
Uneasiness	1504	41.3	34.1
Initiative	1378	35.8	32.9
Short-term memory	1614	26.0	45.5
Interest in surroundings	1410	38.3	34.5
Appearance	1132	40.0	26.9
Vertigo	1463	59.4	31.3
Mobility	1234	35.2	30.5
Migraine	1425	57.7	31.2

of CDP-choline in aiding the evolution of some neuropsychological symptoms such as memory and attention in senile patients without causing side effects. Lozano *et al.* (198) reviewed a series of 2067 elderly patients who had been treated with CDP-choline, at a dose of 300-600 mg/day for two months. The results obtained in terms of the remission and improvement of certain neuropsychological symptoms are shown in Table 11. Palleschi and Capobianco (199) found significant improvement in SCAG and Mini Mental State Examination scores after treatment with CDP-choline in patients with pathological cerebral aging. In a multicenter study with 502 senile patients participating, Schergna and Lupo (200) found that CDP-choline induced significant improvement in attention, behavior, relationships and independence, without any associated side effects. Suryani *et al.* (201) showed that CDP-choline is effective in the treatment of memory deficit in the elderly, attaining significant, progressive improvements in all parameters analyzed (Table 12). CDP-choline improved the scores of senile patients on various scales, such as the Plutchik Scale (202), the Trail Making Test, Randt's Memory Test and the Toulouse-Pieron Attention Test (203, 204).

It has been demonstrated that when administered to healthy adults, CDP-choline acts on the anterior hypophysis, inducing an increase in growth hormone secretion and a decrease in prolactin secretion, due to the induced activation of the dopaminergic system (205, 206). Ceda *et al.* (207) showed that CDP-choline was able to increase growth hormone secretion (both basal secretion and that stimulated by growth hormone releasing hormone) in the elderly, a phenomenon that is found altered in these individuals and, to a greater extent, in patients with cerebral degenerative illnesses.

TABLE 12. Scores for digit repetition, Wechsler's adaptation of the Stanford-Benet logical story test, Bali image memorization test, memory deficit and physical disorders reported by patients before and after treatment with CDP-choline. Values represent mean±SD.

		After treatment		
Tests and manifestations	Initial (n=10)	1 Week (n=10)	2 Weeks (n=10)	3 Weeks (n=6)
Direct repetition of digits	14.6±4.6	19.6±5.6**	20.2±4.5**	22.8±6.0**
Inverse repetition of digits	5.60±4.1	7.30±3.4**	11.3±7.1**	12.1±7.7**
Logical story test	6.10±4.4	9.60±3.8**	12.7±3.7**	13.6±4.8**
Bali image test	5.20±3.2	9.30±3.5**	11.7±3.4**	12.0±2.4**
Memory deficit	2.5±0.9	1.00±0.9*	0.30±0.4**	0.30±0.5**
Physical disorders	2.3±0.9	1.00±0.8*	0.20±0.6**	0.10±0.4**

*p <0.05; **p <0.01 compared to initial scores.

One of the principal causes of cognitive decline in the elderly corresponds to chronic cerebral vascular pathology, also known as cerebral insufficiency, whose maximum degree of clinical expression is vascular dementia. In a double-blind, random, multicenter study versus placebo, the efficacy of CDP-choline was assessed in the treatment of patients with chronic vascular disease (208). This study included 33 patients who received 1 g/day of CDP-choline or saline solution by intravenous perfusion for 28 days. At the end of the treatment period, significant improvement was observed in the Bender-Gestalt Test, the Hamilton Depression Scale, the Parkside Scale, the Neurological Assessment Scale and the Attention Test in the group treated with CDP-choline. Falchi Delitalia *et al.* (209) and Moglia *et al.* (210) observed that this clinical improvement was accompanied by an improvement in the EEG of these patients. Merchan *et al.* (211) showed a progressive improvement in the symptomatology associated with cerebrovascular insufficiency in a group of 40 elderly patients treated with CDP-choline at a dose of 1 g/day, i.m., for 60 days (Table 13).

Agnoli *et al.* (212) carried out a double-blind study on 100 patients with chronic cerebral vascular disease in which they assessed the efficacy of administering CDP-choline at 1 g/day for 28 days, i.v., as compared to placebo. At the end of the treatment period, statistically significant improvements in scores were obtained for the Hamilton Depression Scale and the modified Parkside Behavioral Scale, as well as for the observational and psychometric tests used, leading to the conclusion that CDP-choline improves perceptive motor capacity and attention in these patients, in addition to having a stabilizing effect on behavior. Sinforani *et al.* (213), Motta *et al.* (214) and Rossi and Zanardi (215) obtained very similar results in their respective studies. The best results, from the clinical and behavioral points of view, were observed in patients with diffuse cerebral vasculopathy (216).

Eberhardt and Derr (217) carried out a crossed, double-blind study in order to assess the efficacy and tolerability of CDP-choline in patients with senile cerebral insuffiency. They included 111 patients with an average age of 74.6±6.9 years and a clinical diagnosis of senile cerebral insufficiency. After a wash out period with placebo, patients were placed into two homogenous groups: one of the groups received treatment with 600 mg/day of CDP-choline, p.o., for 5 weeks and placebo for an additional 5 weeks, with a wash out period

TABLE 13. Evolution of symptoms as a function of treatment time.

Symptoms	Day 0	Day 15	Day 30	Day 45	Day 60
Memory deficit	1.58±0.50	1.53±0.51	1.28±0.55**	1.20±0.56#	1.20±0.56#
Concentration difficulty	1.58±0.50	1.58±0.50	1.35±0.48#	1.23±0.42*	1.00±0.60*
Character change	1.10±0.30	1.10±0.30	1.03±0.36#	0.95±0.39*	0.75±0.54*
Depression	1.21±0.41	1.21±0.41	1.04±0.46*	0.88±0.34*	0.75±0.44*
Asthenia	1.50±0.51	1.50±0.51	1.10±0.50	0.88±0.40*	0.70±0.52*
Tension	1.25±0.44	1.25±0.44	1.08±0.41	0.88±0.45*	0.67±0.56*
Anxiety	1.26±0.45	1.22±0.42	1.04±0.47	0.96±0.47	0.83±0.49#
Agitation	1.44±0.51	1.38±0.50	1.19±0.40	1.00±0.00*	0.69±0.48*
Confusion	1.14±0.38	1.14±0.38	1.00±0.58	1.00±0.58**	0.57±0.53**
Emotional lability	1.45±0.50	1.43±0.50	1.10±0.55**	0.88±0.52*	0.70±0.56*
Visual alterations	1.09±0.29	1.09±0.29	0.91±0.29**	0.68±0.48*	0.50±0.51*
Acufens	1.11±0.31	1.11±0.31	0.96±0.43	0.61±0.50*	0.43±0.50*
Vertigo	1.17±0.38	1.14±0.35	0.90±0.41**	0.69±0.47*	0.48±0.51*
Migraine	1.11±0.31	1.07±0.26	0.86±0.45**	0.57±0.50*	0.46±0.51*
Insomnia	1.31±0.47	1.23±0.43	1.04±0.45**	0.73±0.53*	0.54±0.58*

**p <0.004-0.04; #p <0.002-0.004; *p <0.001 compared to initial value.

TABLE 14. Percentages of patients that improved in each of the groups, according to whether they began treatment with CDP-choline or placebo.

Tests and scales	Group I		Group II	
	CDP-choline	Placebo	Placebo	CDP-choline
Numeric repetition	47	31	21	52
Maze	73	69	71	83
Numerical relationships	67	76	67	87
NAS	57	41	44	69
NAB	63	57	48	67
SCAG	80	73	65	83

with placebo between both treatment periods; the other group received the same treatment in reverse order. The tests were carried out at 2, 7, 9 and 12 weeks. CDP-choline as primary treatment significantly improved the clinical status in the six tests used (i.e., numerical memory, mazes, numerical relationships, neuropsychological self-evaluation scale [NAS], gerontopsychological observational scale [NAB] and SCAG), and it brought an additional statistically significant improvement as secondary treatment after placebo, which attained a certain degree of improvement in 5 out of the 6 tests. Intersubject comparisons also showed the superior efficacy of CDP-choline. The percentage of patients that improved in each of the treatment stages is shown for both groups in Table 14. No serious side effects associated with the treatment were found. The authors concluded that these results confirm the efficacy of CDP-choline in the treatment of senile cerebral insufficiency and its excellent tolerability in geriatric patients. These beneficial effects are due to this drug's ability to inhibit the degradation of neuronal membrane phospholipids, to increase plasmatic choline levels and to activate structural phospholipid synthesis and catecholamine synthesis and release. In addition, the effects of CDP-choline in improving test scores were maintained after switching to placebo, which indicates that it is related to the neuronal metabolic process that tends to restore and maintain neuronal functioning.

Chandra (218) recently presented the results of a double-blind study on the treatment of multi-infarct dementia with CDP-choline. The study included 146 patients who were randomly assigned to one of two groups. One of the groups received treatment with CDP-choline, 750 mg/day, i.v., for two months, and the other received saline solution for the same period, with the follow-up continuing for 10 months. At the end of the treatment period, the patients treated with CDP-choline showed significant improvement in Mini Mental State Examination scores, whereas these scores slightly worsened in the group treated with placebo. After 10 months, the patients who had been treated with CDP-choline maintained the observed improvement, whereas the patients in the group treated with placebo continued to worsen (Fig. 25).

Lozano (219) presented the results of a study carried out by the Iberoamerican Group for the Study of Alzheimer's Disease and Longevity, whose goal was to assess the situation and evolution at one year of a group of patients with psycho-organic deterioration of the dementia type, previous diagnosis and classification of its etiological cause (degenerative, vascular or mixed) and oral treatment with CDP-choline. In order to assess the evolution of their dementia for one year, 600 mg/day of CDP-choline, p.o., was administered to 314 patients with an average age of 75.02±7.72 years. The dementia was classified as degenerative in 41.1% of the cases, as vascular in 39.5%, and as mixed in 11.4%. Assessment was carried out at 1, 3 and 12 months using the Burgheon Scale and the Mini Mental State

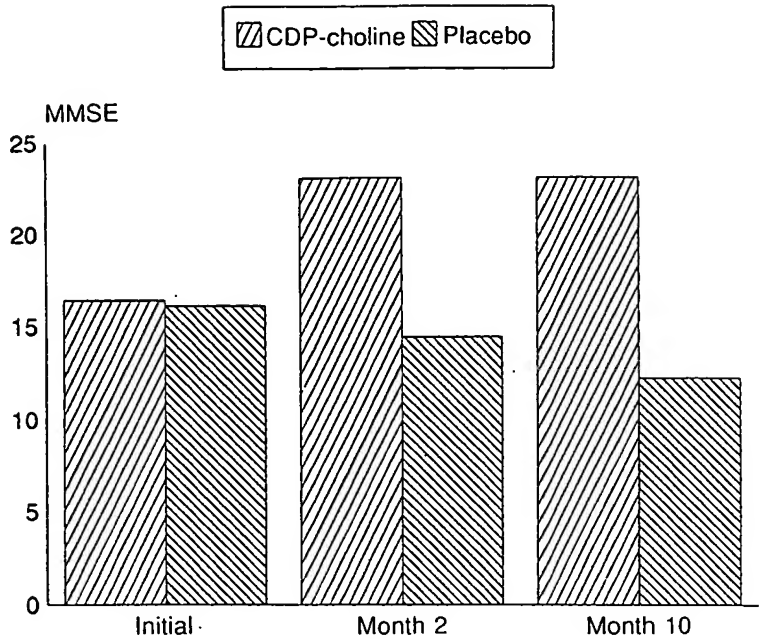

FIG. 25. Scores on the Mini Mental State Examination for 146 patients with multi-infarct dementia with respect to treatment received. Note how in the patients that received CDP-choline, improvement on this scale was maintained up to 8 months after the end of treatment.

Examination. The scores on the Mini Mental State Examination improved significantly in vascular and mixed dementia, and remained stable, with a certain tendency towards improvement in degenerative-type dementia (Table 15). The scores on the Burgheon Scale showed statistically significant improvement in all of the tests and for all three types of dementia (Table 16). These results indicate that CDP-choline has a beneficial effect on the long-term evolution of dementia, and that it is a safe treatment.

Corona *et al.* (220) noted that the beneficial effects of CDP-choline in the treatment of patients with dementia are due, in part, to this drug's ability to improve the activity of serotonergic, dopaminergic and noradrenergic systems, as they showed in a study which assessed the evolution of urinary and CSF levels of monoamine metabolites involved in these systems during the treatment of patients with senile dementia of the Alzheimer's type.

Cacabelos *et al.* (221) carried out a study to evaluate the therapeutic effects of CDP-choline in patients with dementia. In this study, they included 40 patients separated into four groups: 1) 10 healthy elderly subjects; 2) 10 patients with early-onset Alzheimer's disease;

TABLE 15. Evolution in Mini Mental State Examination scores as a function of type of dementia and time of treatment.

Dementia	Start	Day 30	Day 90	Day 360
Degenerative	16.1±4.7	16.2±5.5 ($p<0.03$)	16.5±5.6 (n.s.)	16.8±6.9 ($p<0.009$)
Vascular	16.8±6.0	18.1±6.2 ($p<0.005$)	19.9±6.0 ($p<0.0001$)	20.8±6.5 ($p<0.0001$)
Mixed	18.0±5.2	18.5±5.3 (n.s.)	18.8±5.6 ($p<0.01$)	19.7±5.4 ($p<0.04$)

TABLE 16. Evolution of Bargheon Scale scores as a function of type of dementia and time of treatment.

Dementia	Start	Day 30	Day 90	Day 360
Degenerative	35.4±9.0	32.9±8.0 ($p<0.0001$)	30.9±8.9 ($p<0.0001$)	29.8±10.1 ($p<0.0001$)
Vascular	40.2±10.8	35.5±9.3 ($p<0.0001$)	33.8±9.8 ($p<0.0001$)	32.1±10.6 ($p<0.0001$)
Mixed	42.1±10.8	37.5±9.5 ($p<0.0001$)	35.7±10.7 ($p<0.0001$)	34.9±10.6 ($p<0.0001$)

3) 10 patients with late-onset Alzheimer's disease; and 4) 10 patients with multi-infarct dementia. These patients received treatment with CDP-choline at a dose of 1 g/day, p.o., for 3 months. After this treatment period, they observed a significant improvement in the Mini Mental State Examination scores (Fig. 26) and a significant anti-depressive effect, assessed with the Hamilton Scale for Depression (Fig. 27), in all groups. Furthermore, it was confirmed that in the patients with early-onset Alzheimer's disease, plasma levels of interleukin-1β (IL-1β) were significantly higher than in the other groups, which implies the presence of a neuroimmunological alteration in the physiopathology of Alzheimer's disease. After treatment with CDP-choline, plasma levels of IL-1β normalized, which indicates that this drug possesses some neuroimmunomodulatory activity. In a later phase of the study, this same research team showed that in patients with Alzheimer's disease, CDP-choline improved not only cognitive function, but also cerebrovascular function, measured by transcranial Doppler ultrasonography (222). They demonstrated the neuroimmunological effect of this drug by observing that treatment with CDP-choline reduces plasma histamine levels, which are abnormally elevated in patients with Alzheimer's disease (223).

Recently, Soto *et al.* (224) showed the usefulness of the therapeutic combination of CDP-choline, piracetam and a dihydropyridinic calcium agonist (nicardipine or nimodipine) in the treatment of senile dementia of the Alzheimer's type.

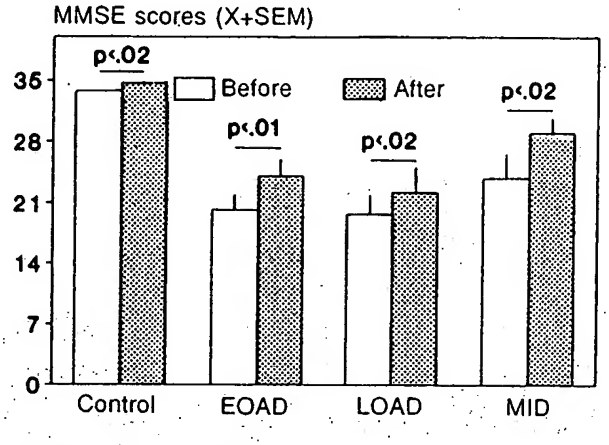

FIG. 26. Effects of CDP-choline on cognitition as evaluated by the Mini Mental State Examination (Spanish version, maximum score = 35 points) in healthy elderly individuals (controls), patients with early-onset (EOAD) or late-onset (LOAD) Alzheimer's disease, and patients with multi-infarct dementia (MID).

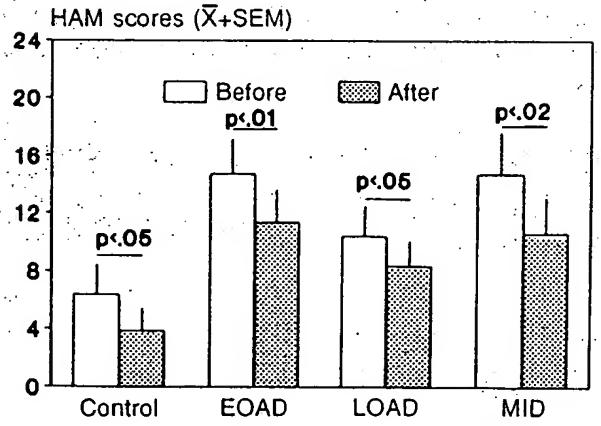

FIG. 27. Antidepressive effects of CDP-choline in healthy elderly individuals and in patients with Alzheimer's disease and multi-infarct dementia, evaluated using the Hamilton depression scale (HAM).

There have been many studies on the use of CDP-choline in the treatment of dementia and cognitive disturbances, and all of them show that this drug produces improvement in cognitive and behavioral aspects. In addition, it has been demonstrated that this drug has beneficial effects on neurophysiological and neuroimmunological changes. All these actions are due to the pleiotropic activity of CDP-choline, which restores neuronal membranes and improves neurotransmission, principally cerebral hemodynamics and neuroimmunological function.

Parkinson's disease

Although levodopa continues to be the main therapeutic agent in Parkinson's disease, its limitations are well known, with the principal one being its progressive loss of effectiveness, often obvious after 3 to 5 years of treatment. Thus, it seems justified to use other drugs which, when combined with levodopa, allow a reduction in its dosage, or which can even be administered as the sole intervention in the first stages of the illness. In this respect, the use of CDP-choline has been tested for its capacity to increase dopamine availability in the striatum and to act as a dopaminergic agonist, as previously analyzed. Since its efficacy has been demonstrated in various experimental models, its use in Parkinson's disease is now accepted (225).

In a double-blind, crossed, comparative study of CDP-choline (600 mg/day for 10 days, i.v.) versus placebo carried out on 28 patients with Parkinson's disease, Ruggieri *et al.* (226) showed that CDP-choline is an effective treatment for this type of patients: it improved the assessment of bradykinesia, rigidity and trembling, as well as the scores on the Webster Scale and the Northwestern University Disability Scale (NUDS). Later, these same researchers (227) obtained very similar results in a broader, follow-up study (Table 17). Subsequently, they tested the effects of CDP-choline on two groups of patients with Parkinson's disease (228); the first group included 28 patients who had not received previous treatment, while the second group included 30 patients who had been already being treated with levodopa and carbidopa for at least two months previously, with the posology having been set at the minimum effective dose. The method followed was the same as that used in previous studies by these same researchers, i.e., a crossed, double-blind, comparative study with placebo. Patients were treated for 20 days with 500 mg/day, parenterally. Clinical assessments were made on days 10 and 20, which coincided with the change in treatment according to the study design. Treatment with CDP-choline yielded statistically significant improvements in both groups of patients on the Webster Scale, on the Northwestern University Disability Scale and in the assessment of bradykinesia. Rigidity also improved in both groups, although this improvement only reached statistical significance in the group of patients who had been treated previously. Trembling also improved in both groups, although it did not reach the desired statistical significance.

Eberhardt *et al.* (229-231) demonstrated that the association of CDP-choline in treatment with levopoda permitted a 50% reduction in the dosage of levodopa, which minimized its associated side effects. This being the case, CDP-choline constitutes a useful alternative in patients who require a reduction in the dose of levodopa. The addition of CDP-choline to treatment with levodopa can also alleviate states of imbalance in the course of parkinsonism (232).

Loeb *et al.* (233) carried out a double-blind, multicenter study with CDP-choline on the treatment of patients with Parkinson's disease. In this study, 65 patients were randomly divided into two groups: one in which 1 g/day of intravenous CDP-choline was added to base treatment for 21 days and another in which placebo was added. All patients continued with their base treatment with levodopa plus carbidopa or benserazide, for a minimum of 8

TABLE 17. Evolution of parkinsonian symptoms and scores on the NUDS and Webster (WRS) scales as a function of treatment sequence.

	Placebo-active sequence			Active-placebo sequence		
	Start	Placebo	Active	Start	Active	Placebo
Bradykinesia	2±0.1	2±0.1	1.4±0.1**	2.6±0.14	1.7±0.13**	2.4±0.19*
Rigidity	2±0.14	2.1±0.12	1.6±0.1**	1.9±0.16	1.4±0.11**	1.9±0.14
Trembling	1.5±0.2	1.6±0.16	1.3±0.15#	1.2±0.19	1.1±0.16	1.2±0.19
NUDS	35.9±0.62	37±1.53#	41.8±1.42**	34.5±1.91	40.5±1.7**	36.5±1.94#
WRS	18.3±0.76	17.8±0.85#	13.4±0.69**	17.7±0.9	13±0.86**	17.1±0.92#

*p <0.05; #p <0.01; **p <0.001 compared to initial scores.

weeks. The authors found significant differences between CDP-choline and placebo in the assessments made on days 14 and 21 of treatment in all of the parameters assessed by the Webster and NUDS scales. In addition, they found that the patients treated with this drug suffered a significant worsening 45 days after suspending treatment, which demonstrates the effectiveness of CDP-choline as a co-treatment with levodopa in patients with Parkinson's disease.

Acosta *et al.* (234) treated 61 patients with Parkinson's disease using CDP-choline; 48 of these patients were already receiving treatment with levodopa. Each patient received two cycles of treatment. In the first cycle, which lasted 10 days, CDP-choline was administered at a dose of 500 mg/day intramuscularly, and subsequently, a second 14-week cycle of treatment followed with the same dosage, this time administered orally. The patients treated with levodopa continued taking it at the same dose during the first period; afterwards, a reduction was attempted. The assessment of Parkinson's symptoms was performed using the Webster Scale. Table 18 shows the average evolution of individual symptoms during the study. Of the patients who received levodopa, 36% improved with the addition of CDP-choline, with the greatest percentage of improvement in bradykinesia, rigidity, posture, walking and balance of the extremities. In the patients who had been treated with levodopa for less than 2 years, it was discovered that the percentage of improvements increased to 42.12%, as compared to 19.08% in the patients with more than 2 years of treatment with levodopa. It was possible to reduce the dose of levodopa from 20 to 100% in 35.3% of the patients with less than 2 years of treatment. In the patients with levodopa treatment of more than 2 years, the dose could be reduced 25-33% in 10% of the cases. The authors concluded that treatment with CDP-choline permits the use of levodopa to be delayed in the early phases of the illness and allows a reduction or maintenance of its dosage in the subjects already being treated.

Cubells and Hernando (235) tested CDP-choline in 30 patients with Parkinson's disease who were already being treated with levodopa. The dose administered was 500 mg/day, i.m., for 2 months, reducing the dose to one-third at the end of the first month of treatment. The evolution of parkinsonian symptomatology, as assessed by the Yahr Scale, showed a moderate improvement in facial expression and manual dexterity, and a substantial improvement in postural stability, motor alterations and bradykinesia after the first month of treatment. A better stabilization in therapeutic response was also observed, with a lower incidence of wearing-off and on-off phenomena, although dyskinesia increased. Upon reducing the dose of levodopa during the second month of study, clinical improvement was maintained and the incidence of dyskinesias was reduced. After one month of treatment with CDP-choline, the changes in various electrophysiological parameters, determined using an original technique of the authors, revealed a recovery from hypo-

TABLE 18. Results of treatment as assessed by the Webster Scale, expressed as mean±SD in all cases (n=61). Percentage of patients who improved is shown for each subcategory.

Webster Scale	Day 1	Day 10	Day 98	% Improvements
Bradykinesia	1.58±0.73	1.38±0.73*	1.15±0.69#	40.7
Rigidity	1.40±0.78	1.27±0.74	1.01±0.72*	41.1
Posture	1.31±0.85	1.13±0.83*	1.05±0.71#	41.5
Balance of upper extremities	1.85±0.96	1.64±0.95*	1.30±0.89#	47.5
Walking	1.41±0.11	1.21±0.10*	1.04±0.72*	50.0
Trembling	1.08±0.85	0.90±0.80*	1.00±0.78*	28.9
Facial expression	1.23±0.58	1.11±0.57*	0.92±0.55#	30.5
Seborrhea	0.65±0.67	0.59±0.66*	0.67±0.61	26.8
Speech	0.90±0.69	0.73±0.62*	0.73±0.59#	29.2
Autonomy	1.01±0.69	0.94±0.67	0.87±0.61*	23.5
Total	12.42±7.91	10.9±6.67	9.74±6.87	35.95

*p <0.05; #p <0.001 compared to day 1.

reflexia and hypotonia, as well as a great improvement in active muscular contraction, diminished muscular fatigue and an evident recovery of contractile speed, a parameter which was found to be very diminished upon initiating treatment with CDP-choline. Plasma levels of the biochemical parameters analyzed and their changes after the first month of treatment with CDP-choline are recorded in Table 19. The authors consider the increase in plasmatic levodopa concentration to be so significant that it cannot be interpreted as being solely due to an increased release of the dopamine stored in presynaptic vesicles; thus, they assume that CDP-choline acts on the dopamine synthesis mechanism by acting on the tyrosine hydroxylase enzymatic system. Furthermore, the increase in dopamine receptors quantified in the lymphocytes is indicative, according to the authors, of the enhancing role of CDP-choline on the availability of postsynaptic dopamine receptors.

Martí-Massó and Urtasun (236) analyzed the effects of CDP-choline in 20 patients with Parkinson's disease who had been receiving levodopa treatment for more than 2 years. These patients were administered 1 g/day of CDP-choline for 15 days, i.m., later continuing with half this dose for an additional 15 days. A progressive improvement in symptomatology was obtained. Thus, on the Columbia University Scale, a global improvement of 4.16% was obtained at 15 days, and of 7.26% at the end of treatment, with noteworthy partial improvements in walking, turnover time in bed and writing time. In the assessment completed by relatives, a noteworthy improvement was obtained in agility, walking and the general state of the patient.

García-Mas *et al.* (237) carried out a study with quantified electroencephalography (EEGq) using fast Fourier transform in two groups of patients with idiopathic Parkinson's disease, with one of these groups evidencing cortical cognitive decline. Study of the specific EEGq indices permitted some differential parameters to be established between the two groups (i.e., those with or without cortical deterioration). Differences were found with respect to the global potentials of alpha and delta frequencies, the alpha/theta index, posterior activities, the anteriorization index of delta and alpha frequencies and, finally, the spacialization index of alpha frequency. The intravenous administration of 3 g of CDP-choline in these patients attained a global increase in the potentials corresponding to posterior frequencies, especially alpha frequency, which is a cognitive activity marker in dementia processes.

TABLE 19. Mean plasma levels (mean±SD) for several biochemical parameters related to levodopa synthesis and metabolism, before and after the first month of treatment with CDP-choline.

Biochemical parameters	Pre-treatment	Post-treatment	*p*
DOPA (ng/ml)*	781.5±302.5	1146.0±403.5	<0.001
3-*O*-methyl dopa (mg/ml)*	10.48±4.37	10.56±4.31	ns
HVA (ng/ml)*	276.1±118.4	666.0±156.1	<0.001
Tyrosine (mg/ml)*	5.25±2.35	5.33±2.26	ns
Lymphocytic dopaminergic receptors**	1.77±0.79	3.93±1.72	<0.01

*Samples extracted 2 hours after administering levodopa; **samples extracted 1 hour after administering levodopa. The binding constant for ^{3}H-spiroperidol is expressed in nanomolar concentrations.

From the studies examined here, it can be asserted that CDP-choline is an effective treatment for Parkinson's disease, both in patients previously untreated and in those who are already receiving treatment with levodopa, in whom it permits a reduction in the dosage. In patients with Parkinson's disease and cognitive decline, CDP-choline tends to normalize the primary altered electrophysiological parameters.

Safety

Dinsdale *et al.* (238) administered CDP-choline to 12 healthy volunteers in two oral-dose regimens repeated after a short time (600 mg/day and 1 g/day) for each of 5 consecutive days, comparing the results with the corresponding administration of placebo. The only adverse effects noted were transient migraines, which occurred in 4 and 5 subjects with low and high doses, respectively; only one of the subjects receiving placebo suffered migraines. The results of the hematological and clinical analyses did not show any abnormality associated with the administration of CDP-choline, nor did the ECGs or EEGs register clinically significant changes. The empirical neurological tests, tendonary reflexes, arterial pressure and heart rate were not affected by any dosages or by placebo.

In addition, its excellent tolerability in healthy individuals, demonstrated in the previous study, all authors of clinical testing in which CDP-choline has been used and which appear in this article agree on the excellent safety of this drug due to the total absence of serious side effects. On some occasions, digestive intolerance has appeared and, occasionally, excitability and uneasiness in the first days of treatment. With this in mind, Lozano (239) monitored a study of CDP-choline's efficacy and safety in 2817 patients of all ages, with a predominance of those between 60 and 80 years, who presented with various neurological processes, for the most part cognitive disturbances of varying etiology. The duration of treatment with CDP-choline varied from 15 to 60 days, and the mean dosis administered was 600 mg/day, p.o. Colateral effects associated with CDP-choline treatment were found in only 5.01% of the patients; the most frequent was digestive intolerance (3.6%). It was not necessary to interrupt treatment due to side effects attributable to the use of CDP-choline in any of the cases.

In conclusion, the tolerability of CDP-choline is excellent and the appearance of side effects attributable to this drug is infrequent. In any case, these side effects are never serious and primarily consist of gastrointestinal discomfort and uneasiness.

CONCLUSIONS

Cytidine 5´-diphosphocholine, CDP-choline or citicoline, is an essential intermediate in the biosynthetic pathway of structural phospholipids of the cell membranes especially of phosphatidylcholine. Upon oral or parenteral administration, CDP-choline releases its two principle components, citidine and choline. When administered orally, it is absorbed almost

completely, and its bioavailability is approximately the same as when administered intravenously. Once absorbed, the cytidine and choline disperse widely throughout the organism, crossing the blood-brain barrier and reaching the central nervous system (CNS), where they are incorporated into the phospholipid fraction of the membrane and microsomes. CDP-choline activates the biosynthesis of structural phospholipids in the neuronal membranes, increases cerebral metabolism and acts on the levels of various neurotransmitters. Thus, it has been experimentally proven that CDP-choline increases noradrenaline and dopamine levels in the CNS. Due to these pharmacological activities, CDP-choline has a neuroprotective effect in situations of hypoxia and ischemia, as well as improving learning and memory performance in animal models of brain aging. Furthermore, it has been demonstrated that CDP-choline restores the activity of mitochondrial ATPase and of membranal Na^+/K^+ ATPase, inhibits the activation of phospholipase A_2, and accelerates the reabsorption of cerebral edema in various experimental models. CDP-choline is a safe drug, as toxicological tests have shown; it has no serious effects on the cholinergic system and it is perfectly tolerated. These pharmacological characteristics, combined with CDP-choline's mechanisms of action, suggest that this drug may be suitable for the treatment of cerebral vascular disease, head injury of varying severity and cognitive disorders of diverse etiology. In studies carried out on the treatment of patients with head injury, CDP-choline accelerated the recovery from post-traumatic coma and recuperated walking ability, achieved a better final functional result and reduced the hospital stay of these patients, as well as improving the cognitive and memory disturbances observed after a less severe head injury, which constitute the disorder known as postconcussion syndrome. In the treatment of patients with acute cerebral vascular disease of the ischemic type, CDP-choline accelerated the recovery of consciousness and motor ability, attaining a better final result and facilitating the rehabilitation of these patients. The other important use for CDP-choline is in the treatment of senile cognitive impairment, whether it be secondary to degenerative diseases (e.g., Alzheimer's disease) or to chronic cerebral vascular disease. In patients with chronic cerebral ischemia, CDP-choline improves scores on cognitive evaluation scales, while in patients with senile dementia of the Alzheimer's type, it slows the disease's evolution. Beneficial neuroendocrine, neuroimmunomodulatory and neurophysiological effects have been described. CDP-choline has also been shown to be effective as co-therapy for Parkinson's disease. No serious side effects have been found in any of the groups of patients treated with CDP-choline, which demonstrates the safety of treatment with this drug.

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